nature neuroscience

βCaMKII controls the direction of plasticity at parallel fiber–Purkinje cell synapses

Geeske M van Woerden[1,5], Freek E Hoebeek[1,5], Zhenyu Gao[1], Raghavendra Y Nagaraja[1], Casper C Hoogenraad[1], Steven A Kushner[2], Christian Hansel[1,4], Chris I De Zeeuw[1,3] & Ype Elgersma[1]

We found that βCaMKII, the predominant CaMKII isoform of the cerebellum, is important for controlling the direction of plasticity at the parallel fiber–Purkinje cell synapse; a protocol that induced synaptic depression in wild-type mice resulted in synaptic potentiation in *Camk2b* knockout mice and vice versa. These findings provide us with unique experimental insight into the mechanisms that transduce graded calcium signals into either synaptic depression or potentiation.

The ability to strengthen and weaken synaptic connections is essential for memory formation. According to the Bienenstock, Cooper and Munro model of synaptic plasticity, the magnitude and direction of synaptic weight changes are not only affected by the temporal pattern of calcium influx, but also by the ability of the synapse to change the modification threshold for synaptic depression and potentiation[1]. However, the precise mechanisms by which a synapse controls the direction of synaptic weight change are still poorly understood.

Calcium/calmodulin-dependent kinase type II (CaMKII) is important for transducing neuronal calcium signals. The CaMKII holoenzyme is a heteromeric protein complex composed of a cell type–dependent ratio of αCaMKII and βCaMKII isoforms. In the adult hippocampus and neocortex, αCaMKII is the predominant isoform[2], where its quantity and autophosphorylation status determine the amplitude and induction threshold of long-term potentiation (LTP)[3–5]. In contrast, αCaMKII is required for long-term depression (LTD), but not LTP, at cerebellar parallel fiber–Purkinje cell synapses[6]. Notably, although βCaMKII is the most prominent cerebellar isoform[2], its function at this synapse is unknown. In addition, although *in vitro* studies have shown that changes in the αCaMKII to βCaMKII ratio have opposing effects on unitary synaptic strength[7], the extent to which changes in the subunit ratio would affect synaptic plasticity *in vivo* is unknown.

To elucidate the role of βCaMKII in synaptic plasticity, we generated a *Camk2b* knockout mouse by inserting the neomycin resistance gene in the *Camk2b* regulatory domain at exon 11 (**Supplementary Fig. 1** and **Supplementary Methods** online). *In situ* hybridization and semi-quantitative RT-PCR analysis of these mice showed that the full-length

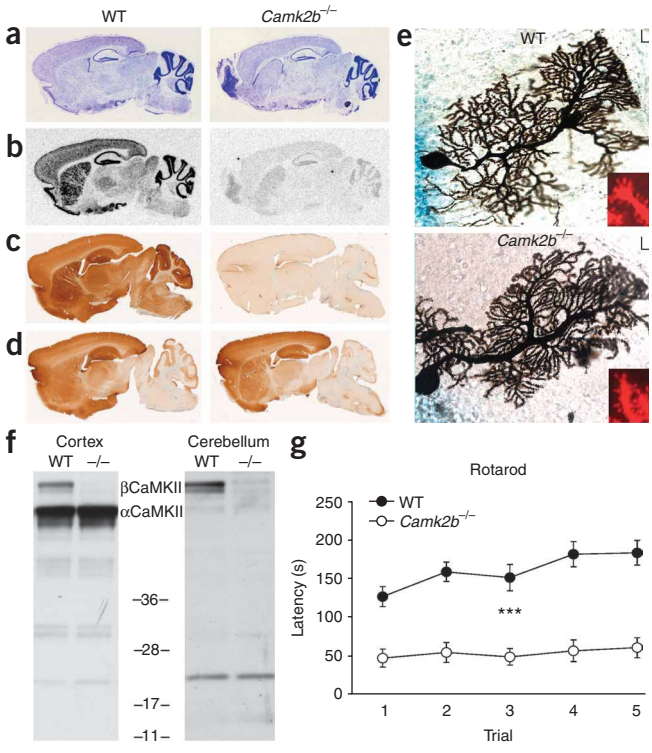

Figure 1 Basal characterization of the *Camk2b*$^{-/-}$ mutant. (**a**) Thionin staining showed no apparent morphological changes in *Camk2b*$^{-/-}$ brains. WT, wild type. (**b**) *In situ* analysis shows decreased levels of 3′ UTR *Camk2b* mRNA in *Camk2b*$^{-/-}$ mutant mice, indicating that gene disruption was successful. (**c**,**d**) Immunocytochemistry using antibodies specific to αCaMKII and βCaMKII isoforms showed a complete loss of βCaMKII immunoreactivity in *Camk2b*$^{-/-}$ brains (**c**) that was not compensated for by upregulation of αCaMKII immunoreactivity (**d**). (**e**) Analysis of Purkinje cell morphology using Golgi-Cox staining and diOlistic labeling (inset) found no differences in arborization and spine density, neck length and head width (see **Supplementary Fig. 4** for quantification). Scale bar represents 10 μM. (**f**) Western blot analysis of wild-type and mutant cortex and cerebellum using an N-terminal pan-CaMKII antibody did not detect a truncated βCaMKII product in *Camk2b*$^{-/-}$ mutants. (**g**) Motor performance assessed by an accelerating rotarod. *Camk2b*$^{-/-}$ mice ($n = 16$) stayed on the rod for a significantly shorter period than wild-type littermates ($n = 16$) and did not increase their performance on training (effect of genotype, $P < 0.005$; interaction training × genotype, $P < 0.05$; repeated measures ANOVA). The latency until the mice fell from the rod is shown. All animal procedures were approved by a Dutch Ethical Committee for animal experiments.

[1]Departments of Neuroscience and [2]Psychiatry, Erasmus University Medical Center, Rotterdam, The Netherlands. [3]Netherlands Institute for Neuroscience, Royal Academy of Sciences (KNAW), Amsterdam, The Netherlands. [4]Present address: Department of Neurobiology, University of Chicago, Chicago, Illinois, USA. [5]These authors contributed equally to this work. Correspondence should be addressed to Y.E. (y.elgersma@erasmusmc.nl) or C.I.D.Z. (c.dezeeuw@erasmusmc.nl).

Received 22 January; accepted 6 April; published online 7 June 2009; doi:10.1038/nn.2329

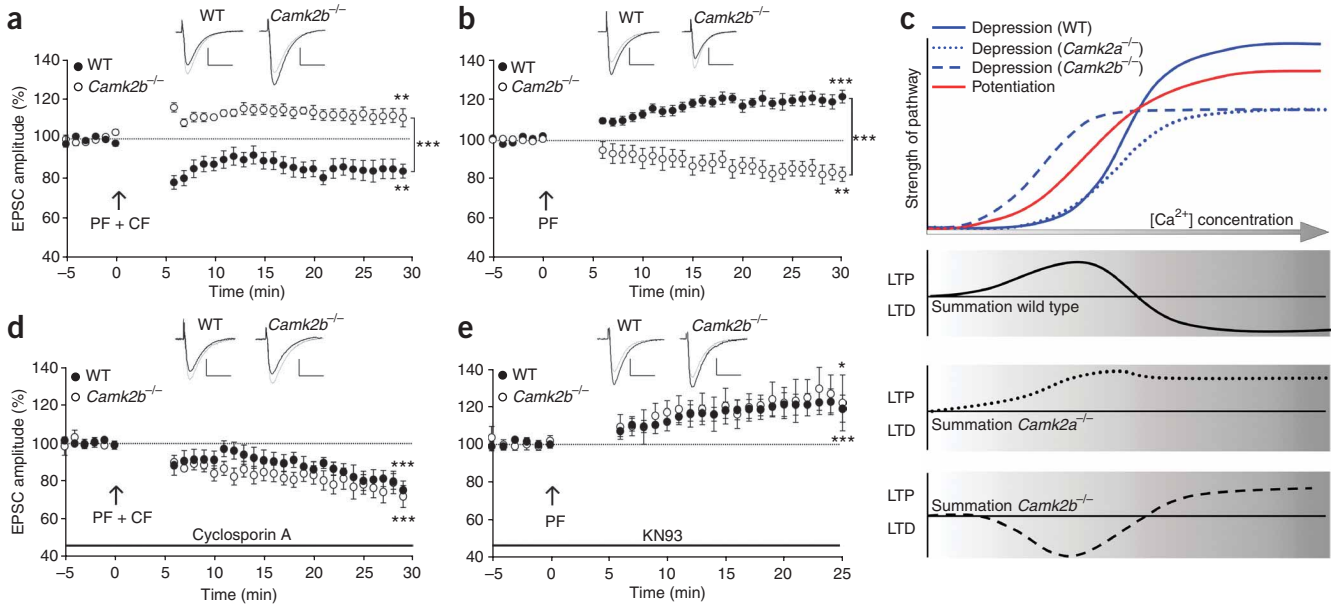

Figure 2 Bidirectional inversion of plasticity at the parallel fiber–Purkinje cell synapse. (**a**) Paired parallel fiber and climbing fiber stimulation (PF and CF, respectively) yielded LTD in wild-type ($n = 12$) Purkinje cells and LTP in *Camk2b*$^{-/-}$ ($n = 11$) Purkinje cells. (**b**) Parallel fiber stimulation alone resulted in LTP in wild-type ($n = 11$) Purkinje cells and LTD in *Camk2b*$^{-/-}$ ($n = 13$) Purkinje cells. (**c**) Schematic model indicating how changes in the CaMKII-driven depression pathway (top) can result in the different plasticity curves observed in wild-type, *Camk2a*$^{-/-}$and *Camk2b*$^{-/-}$ mice (bottom). The model shows that a reduced strength of the kinase-driven depression pathway (down shift), combined with a reduced threshold for calcium activation (left shift), results in an inversion of the plasticity curve in *Camk2b*$^{-/-}$ mice. (**d**) LTD was rescued in *Camk2b*$^{-/-}$ slices in the presence of the PP2B inhibitor cyclosporin A (wild type, $n = 6$; *Camk2b*$^{-/-}$, $n = 13$). (**e**) LTP was rescued in *Camk2b*$^{-/-}$ slices using the CaMKII inhibitor KN93 (wild type, $n = 12$; *Camk2b*$^{-/-}$, $n = 7$). Insets show representative traces before (gray line) and after (black line) tetanization (scale bars represent 100 pA and 10 ms). Error bars represent s.e.m. Asterisks with brackets indicate statistical significance between wild-type and mutant slices (Student's *t* test over the last 5 min). Asterisks without brackets indicate significant difference from baseline (paired *t* test). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.005$.

Camk2b mRNA was lost (**Fig. 1** and **Supplementary Fig. 2** online). In addition, immunostaining and western blot analysis revealed a total loss of βCaMKII immunoreactivity and we could not detect a truncated βCaMKII protein in these mutants (**Fig. 1c**,**f**). The loss of βCaMKII did not cause upregulation of *Camk2a* mRNA or protein (**Fig. 1b** and **Supplementary Fig. 2**). Consequently, total CaMKII activity was significantly reduced, particularly in the cerebellum (reduction of 51% ($P < 0.0001$) for calcium/calmodulin-dependent activity and 41% ($P < 0.005$) for calcium/calmodulin-independent activity; **Supplementary Fig. 1**), suggesting a successful knockout of the *Camk2b* gene.

Although adult *Camk2b*$^{-/-}$ mice showed no differences in survival (measured from weaning till 8 months of age) or general health, they showed pronounced ataxia (**Supplementary Movie 1** online) and severe deficits on the accelerating rotarod test and balance beam test (**Fig. 1g** and **Supplementary Fig. 3** online). Ataxia commonly arises from cerebellar dysfunction and is often observed in mutants with immature or degenerated Purkinje cells. However, thionin staining of brain slices from adult mice revealed no gross differences in overall brain development between wild-type mice and *Camk2b*$^{-/-}$ mutants at the light-microscopy level (**Fig. 1a**). Moreover, Purkinje cells of *Camk2b*$^{-/-}$ mutants did not show significant changes in the complexity of dendritic branching ($P = 0.4$), the density of spines ($P = 0.2$) or Purkinje cell spine maturity (spine neck length ($P = 0.5$) and head width ($P = 0.3$)), indicating that the loss of βCaMKII did not affect gross brain development or the maturation of Purkinje cells *in vivo* (**Fig. 1e** and **Supplementary Fig. 4** online).

Purkinje cells are the sole output of the cerebellar cortex, and plasticity at the parallel fiber–Purkinje cell synapse is generally believed to mediate cerebellar motor learning. To investigate the role of βCaMKII in parallel fiber–Purkinje cell plasticity, we carried out *in vitro* whole-cell recordings of Purkinje cells from adult wild-type and *Camk2b*$^{-/-}$ mice. We tested the ability of Purkinje cells to induce LTD by paired parallel fiber and climbing fiber stimulation at 1 Hz for 5 min[8,9]. This stimulation protocol resulted in significant LTD in wild-type mice ($83 \pm 5\%$, $P < 0.01$), but failed to induce LTD in *Camk2b*$^{-/-}$ mice. In fact, it resulted in significant LTP ($112 \pm 3\%$, $P < 0.01$) in the mutants (**Fig. 2a**), similar to what has been reported for *Camk2a*$^{-/-}$ mice[6]. These results suggest that a critical amount of both αCaMKII and βCaMKII is required for the induction of cerebellar LTD.

We next tested the role of βCaMKII in cerebellar LTP by applying the same parallel fiber stimulus in the absence of climbing fiber stimulation[10]. This postsynaptic form of LTP requires lower calcium transients than LTD induction[9,10]. Using this protocol, we obtained significant LTP in wild-type Purkinje cells ($120 \pm 3\%$, $P < 0.001$), but *Camk2b*$^{-/-}$ Purkinje cells showed robust LTD ($84 \pm 4\%$, $P < 0.01$; **Fig. 2b**). To the best of our knowledge, this is the first report of a genetic or pharmacological manipulation that bidirectionally inverts the polarity of changes in synaptic strength.

Even though the plasticity induced by our protocols is known to be expressed postsynaptically[9–12], the observed changes in synaptic plasticity could potentially result from additional presynaptic changes. However, *Camk2b*$^{-/-}$ mutants showed no difference in basal excitatory synaptic transmission, and Purkinje cells were innervated by a single climbing fiber (**Supplementary Figs. 5** and **6** online), suggesting that the bidirectional inversion of parallel fiber–Purkinje cell plasticity was indeed caused by changes in postsynaptic plasticity.

To better understand how the loss of βCaMKII can result in a bidirectional inversion of plasticity at the parallel fiber–Purkinje cell

synapse, we propose the following plasticity rules that collectively provide a model that fully accounts for the observed data. First, the synaptic depression and potentiation pathways are independent, competing processes driven by kinases and phosphatases, respectively[9,10,12], with each being regulated by a distinct sensitivity for calcium. LTP is generated when phosphatase activity outweighs kinase activity and LTD is generated when kinase activity outweighs phosphatase activity (**Fig. 2c** and **Supplementary Fig. 7** online). Second, because LTP induction is not affected by deleting the *Camk2a* gene[6] or by inhibiting CaMKII kinase activity[11], we assume that αCaMKII and βCaMKII activity contributes to only the synaptic depression pathway (**Fig. 2c** and **Supplementary Fig. 7**). Thus, the loss of either αCaMKII or βCaMKII selectively decreases the depression driving force, and patterns of stimulation that normally produce LTD will therefore yield LTP in the *Camk2a*$^{-/-}$ mutant[6] or *Camk2b*$^{-/-}$ mutant (**Fig. 2a**). Consequently, we predicted that blocking the opposing potentiation pathway by inhibiting calcium-sensitive protein phosphatase 2B (PP2B, calcineurin) activity[12] should restore LTD induction in the *Camk2b*$^{-/-}$ mutant. Indeed, the addition of cyclosporin A during LTD induction yielded a significant induction of LTD in the *Camk2b*$^{-/-}$ mutant ($P < 0.005$; **Fig. 2d**). This indicates that the PP2B-driven synaptic potentiation pathway is in direct competition with the CaMKII-driven synaptic depression pathway and that a loss of βCaMKII activity can be compensated for by inactivating PP2B activity.

Although the aforementioned plasticity rules readily explain the shift from LTD to LTP, as seen in the *Camk2a*$^{-/-}$ (ref. 6) and *Camk2b*$^{-/-}$ mutants, they are not sufficient to explain why an LTP-inducing protocol results in LTD in *Camk2b*$^{-/-}$ mutants. An inversion of the plasticity curve, as observed in the *Camk2b*$^{-/-}$ mutant, can be achieved only if the depression driving force in *Camk2b*$^{-/-}$ mutants is shifted to the left (indicated in **Fig. 2c**), reflecting a lower threshold for activation of this pathway. As αCaMKII is a major driver of the depression pathway[6], this model would predict that activation of αCaMKII is facilitated in the *Camk2b*$^{-/-}$ mutant, leading to precocious induction of the LTD pathway under low-calcium conditions. If this is true, the presence of a CaMKII inhibitor under LTP-inducing conditions should rescue the LTP deficits in the *Camk2b*$^{-/-}$ mutant (**Fig. 2c** and **Supplementary Fig. 7**). This was indeed the case; the addition of the CaMKII inhibitor KN93 restored LTP in *Camk2b*$^{-/-}$ slices to wild-type levels (**Fig. 2e**). These results indicate that the induction of the LTD pathway under low-calcium conditions in the *Camk2b*$^{-/-}$ mutant is caused by the inadvertent activation of αCaMKII.

What could be the cause of the reduced threshold of αCaMKII activation in *Camk2b*$^{-/-}$ mutants? Notably, the reduced threshold for αCaMKII activation was not caused by increased expression of αCaMKII (**Fig. 1** and **Supplementary Fig. 8** online). In addition, there was not a strong increase in basal levels of autonomously activated αCaMKII, as the reduction of calcium-dependent activity was not significantly different from the reduction of calcium-independent activity ($P = 0.5$), and we observed no increase in T286P-αCaMKII (**Supplementary Figs. 1** and **8**). Moreover, the competitive CaMKII inhibitor KN93, which rescued the LTP deficit, could not inhibit αCaMKII if it was already in the autonomously active state, arguing strongly against high basal levels of autonomously active αCaMKII in *Camk2b*$^{-/-}$ mutants.

A more probable explanation comes from the biochemical differences between αCaMKII and βCaMKII. βCaMKII contains an additional domain that enables βCaMKII to cluster the entire CaMKII holoenzyme to F-actin[13–15]. Because all cerebellar αCaMKII is associated with βCaMKII in wild-type mice (**Supplementary Fig. 8**), the loss of βCaMKII will cause a loss of αCaMKII clustering to F-actin, potentially increasing the availability of CaMKII holoenzymes[13,15]. In addition, elegant *in vitro* experiments have shown that the translocation time to the postsynaptic density is fourfold shorter for αCaMKII homo-oligomers than for mixed αCaMKII/βCaMKII hetero-oligomers, and 24-fold shorter than for βCaMKII homo-oligomers[15]. Such a substantial reduction in the translocation time of CaMKII holoenzymes could well explain the reduced threshold for CaMKII activation in the *Camk2b*$^{-/-}$ mutants.

Our results indicate that *Camk2b* is not an essential gene for overall brain development or Purkinje cell maturation, but is instead important for motor coordination and cerebellar plasticity. Although we cannot rule out that these defects are potentially caused by developmental changes, the fact that we were able to rescue both the LTD and LTP deficits by pharmacology suggests a direct role for βCaMKII in the regulation of bidirectional synaptic plasticity at the parallel fiber–Purkinje cell synapse. Specifically, we found that parallel fiber–Purkinje cell plasticity was regulated by a balanced and integrated coordination of kinase and phosphatase activity and that βCaMKII modulated the polarity of synaptic plasticity in two functionally distinct ways; similar to αCaMKII[6], βCaMKII activity was required for driving the synaptic depression pathway under high-calcium conditions. Under low calcium conditions, however, βCaMKII prevented activation of this pathway. Overall, these findings provide us with an insight into the molecular mechanisms governing the rules for bidirectional synaptic plasticity.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

We are grateful to D. Andreev, G. Buitendijk, P. Plak, A. Belmeguenai, D. Jaarsma, M. Vandeputte, M. Elgersma, E. Haasdijk, H. Goedknegt, B. Dortland, M. Aghadavoud and J. Kongasan for technical assistance and to G. Borst and R. Colbran for stimulating discussions. This work was supported by grants from the Netherlands Organization for Scientific Research (C.H., C.I.D.Z. F.E.H., and Y.E.), Neuro-BSIK (C.I.D.Z. and Y.E.), EEC-SENSOPAC and Prinses Beatrix Fonds (C.I.D.Z.).

AUTHOR CONTRIBUTIONS

G.M.v.W. generated the mutant and conducted the molecular and behavioral characterization, F.E.H., Z.G. and R.Y.N. conducted the electrophysiology experiments, C.C.H. performed the diOlystic labeling, Y.E., C.I.D.Z. and C.H. supervised the project, and S.A.K. and Y.E. generated the models. Y.E., S.A.K., G.M.v.W. and F.E.H. wrote the manuscript.

Published online at http://www.nature.com/natureneuroscience/
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions/

1. Bienenstock, E.L., Cooper, L.N. & Munro, P.W. *J. Neurosci.* **2**, 32–48 (1982).
2. Erondu, N.E. & Kennedy, M.B. *J. Neurosci.* **5**, 3270–3277 (1985).
3. Silva, A.J., Stevens, C.F., Tonegawa, S. & Wang, Y. *Science* **257**, 201–206 (1992).
4. Elgersma, Y. *et al. Neuron* **36**, 493–505 (2002).
5. Giese, K.P., Fedorov, N.B., Filipkowski, R.K. & Silva, A.J. *Science* **279**, 870–873 (1998).
6. Hansel, C. *et al. Neuron* **51**, 835–843 (2006).
7. Thiagarajan, T.C., Piedras-Renteria, E.S. & Tsien, R.W. *Neuron* **36**, 1103–1114 (2002).
8. Tanaka, K. *et al. Neuron* **54**, 787–800 (2007).
9. Coesmans, M., Weber, J.T., De Zeeuw, C.I. & Hansel, C. *Neuron* **44**, 691–700 (2004).
10. Lev-Ram, V., Wong, S.T., Storm, D.R. & Tsien, R.Y. *Proc. Natl. Acad. Sci. USA* **99**, 8389–8393 (2002).
11. Kakegawa, W. & Yuzaki, M. *Proc. Natl. Acad. Sci. USA* **102**, 17846–17851 (2005).
12. Belmeguenai, A. & Hansel, C. *J. Neurosci.* **25**, 10768–10772 (2005).
13. Shen, K., Teruel, M.N., Subramanian, K. & Meyer, T. *Neuron* **21**, 593–606 (1998).
14. Okamoto, K., Narayanan, R., Lee, S.H., Murata, K. & Hayashi, Y. *Proc. Natl. Acad. Sci. USA* **104**, 6418–6423 (2007).
15. Shen, K. & Meyer, T. *Science* **284**, 162–166 (1999).