Potential role of Synaptopodin in hippocampal synaptic plasticity

BY GIULIA C. S. GOOD STEPHANI '01

Introduction

Long Term Potentiation

Understanding the mechanisms of memory is one of neuroscience's age-old problems. Many neurobiologists concerned with the fundamental physical or chemical processes involved in the information storage investigate the regulation of synaptic strength. In particular, the hippocampus has been the site of intensive research due to its established role in learning and memory. Long term potentiation (LTP) of synaptic transmission in the hippocampus is the primary experimental model for investigating the synaptic basis of learning and memory in vertebrates (Bliss and Collingridge 1993). LTP refers to the long lasting enhancement of the effectiveness of the synaptic transmission that follows brief trains of high-frequency stimulation (Kandel et al 2000). LTP results in long-lasting changes in the connections between neurons, which are speculated to be the cellular basis for information storage in the brain (Hebb 1949). LTP is expressed by a persistent increase in the size of the excitatory response to a given stimulus (Bliss and Collingridge 1993). Calcium influx into the postsynaptic cell is essential for the induction of LTP; however, the subsequent secondary processes and the final changes made to maintain synaptic strengthening are much less clear.

Bliss and Collingridge hypothesize that the expression of synaptic potentiation most likely involves both pre- and postsynaptic mechanisms, respectively leading to an increase in neurotransmitter release and to a change in number or properties of the ion channels, which mediate synaptic transmission (1993). A number of studies have sought to show that activity-induced changes in the morphology or number of spines may also contribute to changes in synaptic efficiency (Luscher et al. 2000; Toni et al. 1999; Engert and Bonhoeffer 1999). Through a combination of two relatively new techniques, two-photon laser scanning microscopy and local micro-superfusion, recent work has shown that spines do change their morphology when synapses are functionally strengthened (Yuste and Bonhoeffer 2001; Engert and Bonhoeffer 1999). In addition, Yuste and Bonhoeffer discovered that LTP in a hippocampal neuron is accompanied by the emergence of new spines (2001). Although more experiments on the precise nature of these changes are necessary, the data provide strong evidence that both physiological and structural changes play an important role when neurons change the efficacy of their connections.

In this study, acute slices of the hippocampus of knock-out mice brains are compared to wildtype slices and used to examine the role of the synaptopodin protein in the enhancement of synapses as it occurs in hippocampal LTP. For our studies, LTP is a convenient measure to compare changes in synaptic efficacy in wild type mice and synaptopodin knock-out mice. In doing so, we hope to better understand the role synaptopodin may have on learning and memory.

Synaptopodin

Dendritic spines are dynamic structures that rapidly remodel their shape and size. These morphological adaptations are regulated by changes in synaptic activity and result from rearrangements of the postsynaptic cytoskeleton. Recently, a novel actin-associated molecule, synaptopodin, was cloned and characterized (Mundel et al. 1997). Synaptopodin is a proline-rich, actin-associated protein. This molecule shows no significant homology to any other known protein in the brain. The protein is pres-

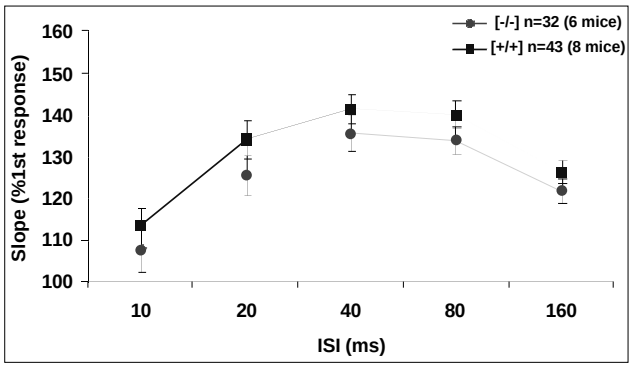

Figure 1: Paired-pulse facilitation (PPF) shown in wild type [+/+] and synaptopodin knock-out mice [-/-] taken at intervals of 10, 20, 40, 80, and 160ms. Knock-out mice demonstrate PPF, but at lower values on average than wild type mice for all time intervals.

ent in renal podocytes and in dendritic spines. In the kidney, synaptopodin plays a role in retraction and extension, which is part of podocyte foot processes (Mundel et al. 1997). Synaptopodin synthesis in the telencephalon has been confirmed by in situ hybridization, where synaptopodin mRNA is only found in perikarya of the olfactory bulb, cerebral cortex, striatum, and hippocampus; i.e., the expression is restricted to areas of high synaptic plasticity (Mundel et al. 1997).

Deller et al. analyzed the distribution of synaptopodin in the hippocampal formation (2000). Postembedding immunogold histochemistry found synaptopodin to be exclusively present in dendrites and spines, and within the spine microdomain, synaptopodin was concentrated in the spine neck and in close association with the spine apparatus. Spines in which the spine apparatus was absent were not immunoreactive for synaptopodin (Deller et al. 2000). These findings suggest that synaptopodin may play a role in linking the spine apparatus to actin and may contribute to the morphological change in spine shape considered to be important for the maintenance of synaptic plasticity.

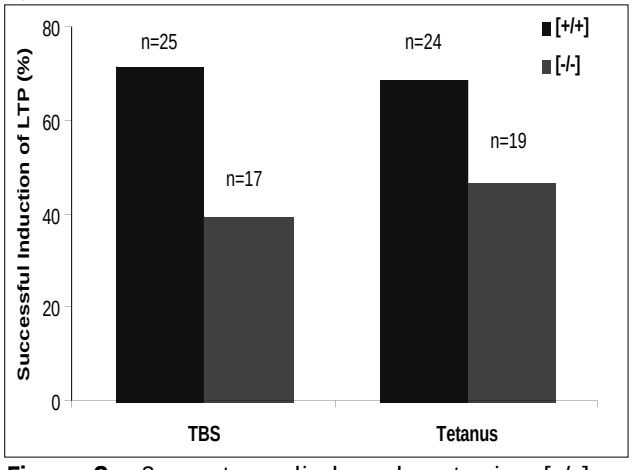

Figure 2a: Synaptopodin knock-out mice [-/-] show a significant reduction in the induction rate of long term potentiation (LTP) when compared to wild type mice [+/+] for both theta-burst and tetanus stimuli, n = number of slices; experiments were conducted using 4 [+/+] mice and 3 [-/-] mice (for a total of 8 [+/+] and 6 [-/-]).

Methods

Slice preparation

All knock-out animals used in this study were generated by Peter Mundel and Rolf Zeller at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. The brains from synaptopodin knock-out and wild type mice (P89 – P139) were quickly removed from the skull and placed in ice cold ACSF (artificial cerebrospinal fluid) containing 124 mM NaCl, 3 mM KCl, 1.25 mM KH2PO4, 2 mM MgSO4, 26 mM NaHCO3, 2.5 mM CaCl2, and 10 mM

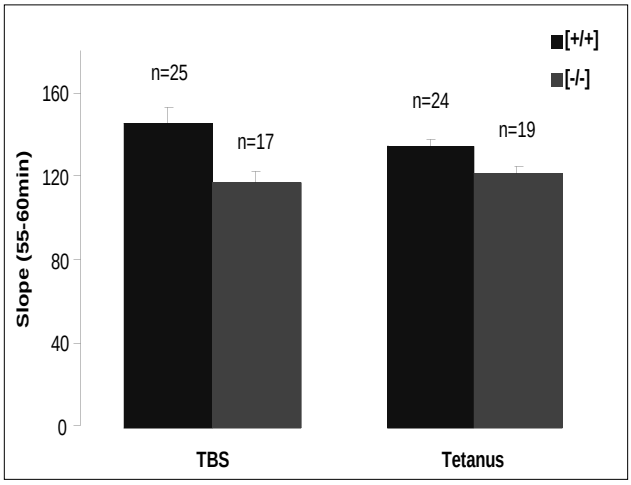

Figure 2b: One hour after stimulus, synaptopodin knock-out mice [-/-] show significantly lower average slope values compared to wild type mice [+/+], n = number of slices; experiments were conducted using 4 [+/+] mice and 3 [-/-] mice (for a total of 8 [+/+] and 6 [-/-]).

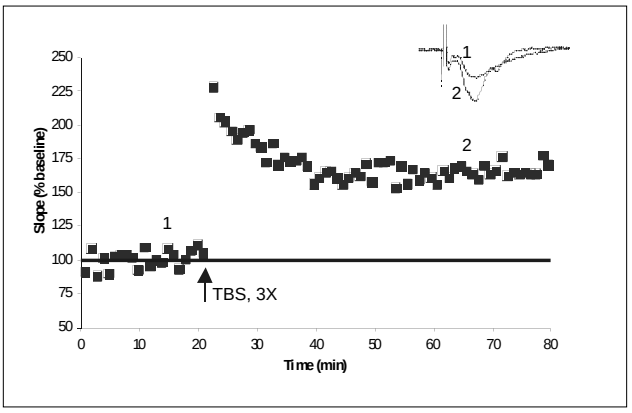

Figure 3a: Single experiment, wild type mouse [+/+]. Average values over six time points. Inserts: original EPSPs before and after TBS.

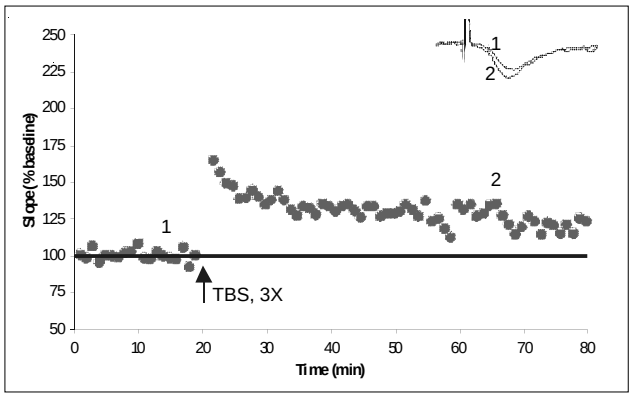

Figure 3b: Single experiment, synaptopodin knock-out mouse [-/-]. Average values over six time points. Inserts: original EPSPs before and after TBS.

Glucose. The hippocampus was dissected from the rest of the brain, and hippocampal transverse slices (400 µm thick) were prepared with a vibratome or an egg slicer. The slices were placed in an incubation chamber at room temperature containing oxygenated ACSF. Following preparation, slices were allowed a one-hour period of recuperation prior to electrophysiological experiments. For each individual experiment one slice was transferred into a 'submerged' recording chamber. Oxygenated ACSF was perfused through the chamber (1ml/min) with a temperature of 32 ± 0.2°C. This slice preparation is the same as that described by Korte et al. in their work looking at the late phase of hippocampal LTP in brain-derived neurtrophic factor (BDNF) knock-out mice (1998).

Electrophysiology

Field excitatory postsynaptic potentials (EPSPs) measured in stratum radium were evoked by stimulation of the Schaffer collateral-commissural afferents; we stimulated at CA3 and recorded at CA1. Extracellular recording electrodes were filled with 3M NaCl. In each experiment, a baseline recording of 20 minutes proceeded the application of high frequency stimulus. LTP was induced using three consecutive theta burst stimuli (TBS) each of 10 x 4 pulses with 200 ms interburst intervals and 10 ms intervals within each pulse. A single tetanus stimulus consists of 3 series of 30 pulses (100 Hz). Each pulse length is 100 µs. Following TBS or tetanus, post stimulation recordings were continued for 60 minutes.

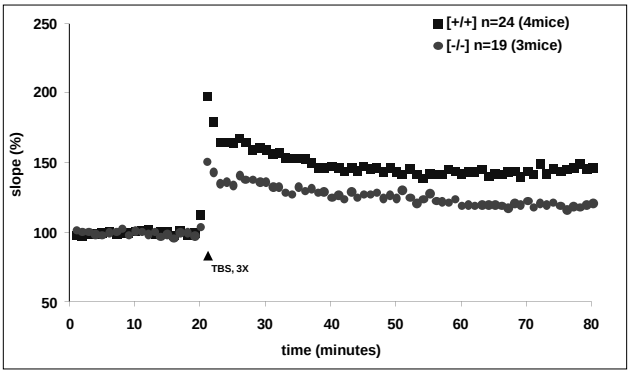

Figure 4: Theta-burst stimulus (TBS) summary graph. Synaptopodin knock-out mice [-/-] show significantly reduced potentiation for all data points recorded after TBS compared to wild type mice [+/+]. Error bars: standard error of mean, n = number of slices.

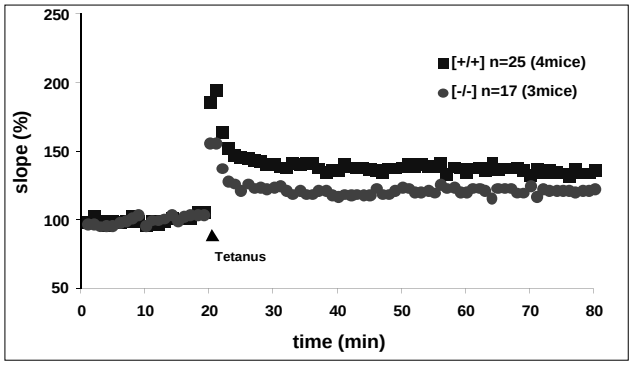

Figure 5: Tetanus stimulus summary graph. Synaptopodin knock-out mice [-/-] show significantly reduced potentiation for all data points recorded after high frequency stimulation compared to wild type mice [+/+]. Error bars: standard error of mean, n = number of slices.

Paired pulse recordings were made at intervals of 10, 20, 40, 80, and 160 ms following the initial pulse. Ensemble averages were constructed using all data points and were aligned with respect to the time of LTP induction. The error bars shown are the standard error of the mean calculated for the entire data set for a given time point.

Results

Synaptopodin knock-out mice demonstrate paired-pulse facilitation (PPF), but at lower values than wild type mice for all measured time intervals (Fig.1). Synaptopodin knock-out mice show a significant reduction in the induction rate of LTP when compared to wild type mice for both the TBS and tetanus stimulus (Fig. 2a). The induction rate of LTP for the TBS-paradigm was 40% and 72% for the knock-out and wild type mice, respectively. For the tetanus stimulus, the induction rate was 47% in the knock-out mice and 69% in the wild type mice (Fig.2a.). One hour after stimulus, synaptopodin knock-out mice show significantly lower average EPSP-slope values than wild type mice. One hour after TBS, and averaged over a five minute interval, the knock-out mice have a slope size for all slices of 118 % (+/-4.8%) of the established baseline, compared to 145% (+/-7.8%) of the baseline in the wild type mice (4 mice, 24 slices [+/+]; 3 mice, 19 slices [-/-]). The difference in percent of baseline is smaller, but still significant, in the tetanus experiments; the knock-out animals have an average slope for all slices of 121%(+/-4.1%) of baseline, compared to 134% (+/-3.6%) in the wild type mice (4 mice, 25 slices [+/+]; 3 mice, 17 slices [-/-]) (Fig.2b.).

Synaptopodin knock-out mice show significantly reduced slope values over all time periods following TBS; data points averaged for each time point for all experiments are shown in Fig.4. Posttetanic-potentiation is lower in knock-out mice, but still visible.

Synaptopodin knock-out mice show significantly reduced slope values over all time periods following tetanus stimulus. Data points averaged for each time point for all experiments are shown in Fig.5.

Conclusions

In this study, activity dependent synaptic plasticity of synaptopodin knock-out mice was compared to wildtype mice and basal synaptic transmission and LTP were analyzed in the CA3-CA1-pathway of the hippocampus. We suggest here that LTP-induced synaptopodin regulates the dynamics of the acitn-based cytoskeleton, which would contribute to the long-lasting change in synaptic morphology associated with the maintenance of synaptic plasticity. Fischer et al. monitored spine movement of living neurons and demonstrated that changes in spine shape can occur within seconds (1998). Our findings lend support to the hypothesis that synaptopodin affects spine motility by bundling actin filaments in the spine neck. This rapid movement of spines is actin-based and primarily involves spine shape and does not affect spine size or number. However, it may be that the rapid de novo formation of spines accompanying synaptic potentiation is an actin-mediated process (Engert and Bonhoeffer 1999). Our study also supports the hypothesis that synaptopodin links the actin cytoskeleton of spines to intracellular calcium stores, i.e., the spine apparatus and the smooth endoplasmic reticulum.

The findings of this neurophysiological study on synaptopodin knock-out mice in combination with earlier work analyzing the distribution of the protein in the hippocampus suggest that the synaptopodin protein may be involved in the actin based plasticity of spines and thus ultimately in learning and the formation of memories. These established physical changes involving the synaptopodin protien may be a way of 'engraving' information in the brain. The implications of this study are highly significant and warrant further study with more synaptopodin knock-out animals, testing on younger

animals, using alternative LTP induction protocols, and running trials of longer duration. In addition, investigation into the precise mechanisms by which synaptopodin contributes to the rearrangement of the actin-based cytoskeleton and influences the change in synaptic shape would help clarify the precise role of the protein. Fully understanding the role of the synaptopodin protein may one day aid medical research in a number of fields including, though not exclusively, learning disabilities, amnesia, Alzheimer's, stroke recovery, epilepsy, and memory loss associated with ageing.

Acknowledgements

Many thanks to Tobias Bonhoeffer, Martin Korte, and Volker Staiger at the Max Planck Institute of Neurophysiology in Munich, Germany for hosting me and for making me feel like part of the team. Thanks as well to the Dartmouth College German and Biological Sciences Departments and the Dickey Center for International Understanding for their support of my work in Germany.

References

Bliss T.V.P. and G.L. Collingridge. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 361: 31-39.

Deller, Th., T. Merten, S.U. Roth, P. Mundel, M. Frotscher. 2000. Actin-associated protein synaptopodin in the rat hippocampal formation: localization in the spine neck and close association with the spine apparatus of principal neurons. Journal of Comparative Neurology. 418: 164-181.

Engert, F. and T. Bonhoeffer. 1999. Dendritic spine changes associated with hippocampal long-term synaptic plasticity. Nature. 399: 66-70.

Fischer, M, S.Kaech, D.Knutti, A. Matus. 1998. Rapid actin-based plasticity in dentritic spines. Neuron. 20L 847-854.

Hebb D.O. The organization of Behavior. A neurophysiological Theory. Wiley: New York, 1949.

Kandel, E.R., J.H. Schwartz, T.M. Jessell. Principles of Neural Science. McGraw-Hill Companies, New York: 2000.

Korte, M., H. Kang, T. Bonhoeffer, E. Schuman. 1998. A role for BDNF in the late-phase of hippocampal long-term potentiation. Neuro Pharmacology. 37: 553-559.

Luscher, C., R.A. Nicoll, R.C. Malenka, D. Muller. 2000. Synaptic plasticity and dynamic modulation of the post-synaptic membrane. Nature Neuroscience. 3: 545-550.

Mundel, Peter, H.W. Heid, T.M. Mundel, M. Kruger, J. Reiser, W. Kriz. 1997. Synaptopodin: An Actin-associated Protein in Telencephalic Dendrites and Renal Podocytes. The Journal of Cell Biology. 139: 193-204.

Toni, N., P.A. Buchs, I. Nikonenko, C.R. Bron, D. Muller. 1999. LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. Nature. 402: 421-425.

Yuste R, Bonhoeffer T, (2001) Morphological changes in dendritic spines associated with long-term synaptic plasticity Annual Review of Neuroscience. 24:1071-1089

ABOUT THE AUTHOR

Giulia C.S. Good Stefani '01 is a Biological Sciences major. She is headed out to Hood River, OR for the summer to work for the Forest Service as a wildland fire fighter. In the fall, she will be working for a non-profit organization in Oakland, CA before heading to Berlin, Germany for a one year internship in the German Parliment.