Neurobiology of Learning and Memory xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Review

Generation of multi-innervated dendritic spines as a novel mechanism of long-term memory formation

Karl Peter Giese [a,*], Wajeeha Aziz [a], Igor Kraev [b], Michael G. Stewart [b]

[a] Department of Basic and Clinical Neuroscience, King's College London, UK
[b] Department of Life Science, Open University, Milton Keynes, UK

ARTICLE INFO

Article history:
Received 21 January 2015
Revised 20 April 2015
Accepted 22 April 2015
Available online xxxx

Keywords:
Hippocampus
Memory
Synaptic plasticity
Synaptic signaling

ABSTRACT

NMDA receptor-dependent long-term potentiation (LTP) at hippocampal CA1 synapses is a well-accepted mechanism underlying long-term memory (LTM) formation. However, studies with mice that lack threonine-286 autophosphorylation of αCaMKII have shown that hippocampal LTM can be formed despite absence of NMDA receptor-dependent CA1 LTP. After multiple training trials, LTM formation in these mutants is linked to the generation of multi-innervated dendritic spines (MIS), a spine that receives typically two presynaptic inputs. PSD-95 overexpression is sufficient for MIS generation and depends on mTOR signaling. LTM that involves MIS generation appears less modifiable upon retrieval in comparison to LTM without MIS generation. Taken together, MIS generation appears to be a novel LTM mechanism after multiple training trials, which may occur in diseases with impaired LTP or conditions affecting negative feedback CaMKII signaling at the synapse.
© 2015 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. In search of cellular mechanisms of long-term memory formation

Mechanistic understanding of long-term memory (LTM) is one of the big challenges in neuroscience. As a cellular mechanism underlying LTM, NMDA receptor-dependent long-term potentiation (LTP) has received a lot of attention. This type of plasticity occurs at hippocampal CA1 synapses after training in relevant memory tasks (Gruart, Munoz, & Delgado-Garcia, 2006; Whitlock, Heynen, Shuler, & Bear, 2006). Further, pharmacologic or molecular biological alteration of NMDA receptor-dependent LTP at CA1 synapses affects LTM formation or storage (Giese, 2012; Martin, Grimwood, & Morris, 2000; Silva, 2003). Recently, the causal link between NMDA receptor-dependent LTP and LTM has been strengthened with an optogenetic approach, where long-term depression (LTD) impairs LTM and LTP induction is sufficient to restore LTM (Nabavi et al., 2014). Whilst this evidence demonstrates that NMDA receptor-dependent LTP is important for LTM, it has also become clear that there is more to LTM than just NMDA receptor-dependent LTP. In particular, investigations with the αCaMKII^{T286A} mouse model have shown that LTM can be formed in the absence of NMDA receptor-dependent LTP at CA1 synapses.

2. The αCaMKII^{T286A} mouse model

The α-isoform of calcium/calmodulin-dependent kinase II (αCaMKII) is the most abundant signaling protein in the postsynaptic density of glutamatergic neurons in the forebrain (Kennedy, 2000). NMDA receptor opening at the postsynaptic membrane leads to activation of αCaMKII. Once activated, αCaMKII autophosphorylates at the so-called autonomy site at threonine-286 (T286). This autophosphorylation prolongs αCaMKII activity for about one minute after LTP induction (Lee, Escobedo-Lozoya, Szatmari, & Yasuda, 2009). αCaMKII^{T286A} mice have a targeted point mutation in the αCaMKII gene that prevents phosphorylation at the autonomy site threonine-286 (Giese, Fedorov, Filipkowski, & Silva, 1998). Thorough electrophysiological investigation have established that the lack of T286 autophosphorylation completely abolishes NMDA receptor-dependent LTP at hippocampal CA1 synapses in adult, but not at neonatal age (Cooke et al., 2006; Giese et al., 1998; Irvine et al., 2011; Radwanska et al., 2011; Villers, Giese, & Ris, 2014; Yasuda, Barth, Stellwagen, & Malenka, 2003). These LTP impairments were demonstrated in hippocampal slices and in anaesthetised mice. However, despite the absence of NMDA receptor-dependent LTP at CA1 synapses, αCaMKII^{T286A} mice can

* Corresponding author at: Centre for the Cellular Basis of Behaviour, James Black Centre, King's College London, Institute of Psychiatry, 125 Coldharbour Lane, London SE5 9NU, UK. Fax: +44 (0)20 7848 3808.
E-mail address: Karl.Giese@kcl.ac.uk (K.P. Giese).

http://dx.doi.org/10.1016/j.nlm.2015.04.009
1074-7427/© 2015 The Authors. Published by Elsevier Inc.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Please cite this article in press as: Giese, K. P., et al. Generation of multi-innervated dendritic spines as a novel mechanism of long-term memory formation. *Neurobiology of Learning and Memory* (2015), http://dx.doi.org/10.1016/j.nlm.2015.04.009

form hippocampus-dependent LTM (Irvine, Vernon, & Giese, 2005; Irvine et al., 2011; Radwanska et al., 2011). This was shown after training in contextual fear conditioning and the passive avoidance task. The lack of T286 autophosphorylation abolishes one-trial LTM formation, but LTM can be formed with massed training. For example, five massed conditioning trials enable contextual fear LTM formation in αCaMKII^{T286A} mice (Irvine et al., 2005, 2011; Radwanska et al., 2011). Importantly, this contextual fear LTM in the absence of T286 autophosphorylation depends on the hippocampus as revealed by post-training lesion experiments (Irvine et al., 2011). Further, immunohistochemical analysis after contextual fear conditioning showed that the αCaMKII^{T286A} mutants do not upregulate Zif268 and NR4a1 in hippocampal area CA1 (Radwanska et al., 2011), immediate-early genes that are required for the late phase of NMDA receptor-dependent LTP (Bridi & Abel, 2013; Jones et al., 2001). Thus, the deficient upregulation of these immediate-early genes indicates that contextual fear conditioning-induced NMDA receptor-dependent LTP is impaired in the αCaMKII^{T286A} mutants. Moreover, immediate-early gene imaging after contextual fear conditioning showed that there is no upregulation in alternate brain regions in the mutants, demonstrating that there is no compensation at the systems level that leads to contextual fear LTM in αCaMKII^{T286A} mice (Radwanska et al., 2011). Taken together, this concludes that in αCaMKII^{T286A} mice hippocampus-dependent LTM can be formed despite complete lack of NMDA receptor-dependent LTP at CA1 synapses. An alternate plasticity mechanism at CA1 synapses appears sufficient for LTM formation in αCaMKII^{T286A} mice.

LTP involves the strengthening of existing synapses. In principle, it is conceivable that synaptogenesis may compensate for impaired LTP to enable LTM formation in the αCaMKII^{T286A} mice. This idea was tested with electron microscopy (EM) analysis (Radwanska et al., 2011). Excitatory synapse numbers in stratum radiatum of area CA1 were compared: (i) before, and at (ii) 2 h and (iii) 24 h after contextual fear conditioning. Both wild-type and αCaMKII^{T286A} mice showed up-regulated synapse numbers at 2 h after conditioning. This synaptogenesis occurred in the mutants despite impaired induction of the immediate-early genes c-Fos, Zif268 and NR4a1 (Radwanska et al., 2011). Thus, local protein synthesis might lead to contextual fear conditioning-induced synaptogenesis (Holt & Schuman, 2013). The contextual fear conditioning-induced increase in synapse numbers is transient as after 24 h there is not net increase in synapse numbers (Radwanska et al., 2011). This finding appears in contradiction with a dendritic spine analysis using Golgi staining, showing increased spine density in hippocampal area CA1 24 h after contextual fear conditioning (Restivo, Vetere, Bontempi, & Ammassari-Teule, 2009). However, this study analyzed spines after a retrieval trial that triggers reconsolidation and which could have induced spinogenesis.

Transient up-regulation in synaptogenesis cannot account for LTM in αCaMKII^{T286A} mice, which was tested much later than 2 h after conditioning (Irvine et al., 2005). However, further EM data analysis revealed that generation of an atypical excitatory synapse type, multi-innervated dendritic spines (MIS) (Fig. 1), correlated specifically with LTM formation in αCaMKII^{T286A} mice and not in wild-type mice (Radwanska et al., 2011).

3. Multi-innervated dendritic spines and long-term memory formation

The vast majority of excitatory synapses in hippocampal area CA1 have a 1:1 relationship between a presynaptic terminal and a dendritic spine. However, in some cases a dendritic spine receives more than one presynaptic input (Fiala, Feinberg, Popov, & Harris, 1998). These structures as shown in Fig. 1 have been termed multi-innervated dendritic spines (MIS) (Nikonenko, Jourdain, & Muller, 2003). In adult hippocampal area CA1, MIS have typically two presynaptic inputs and they represent less than 1% of excitatory synapses (Petrak, Harris, & Kirov, 2005; Radwanska et al., 2011). MIS develop by the attraction of presynaptic terminal(s) onto an existing dendritic spines (Nikonenko et al., 2008; Petrak et al., 2005). In hippocampal slice cultures NMDA receptor activation can induce MIS generation (Nikonenko et al., 2003). Further, PSD-95 overexpression is sufficient to generate MIS (Nikonenko et al., 2008). This overexpression leads to binding of PSD-95 to neuronal nitric oxide synthase (nNOS), which causes production of the retrograde messenger nitric oxide that attracts presynaptic terminal(s) onto the existing dendritic spine with multiple distinct postsynaptic densities (PSDs) (Nikonenko et al., 2008).

Contextual fear conditioning with αCaMKII^{T286A} mice showed for the first time that behavioral experience can induce MIS generation (Radwanska et al., 2011). Training-induced MIS generation was analyzed only in hippocampal area CA1. It remains possible that MIS generation also occurs in other brain areas, such as the amygdala, after contextual fear conditioning of αCaMKII^{T286A} mice. As in hippocampal slice culture experiments, the behaviorally-induced MIS generation in CA1 correlates with PSD-95 protein expression (Radwanska et al., 2011). PSD-95 up-regulation occurs in αCaMKII^{T286A} mice after five massed conditioning trials, but not after a single conditioning trial. Further, PSD-95 up-regulation and the contextual fear LTM in αCaMKII^{T286A} mice depend on mTOR signaling (Radwanska et al., 2011). Taken together, this suggests that MIS generation enables contextual fear LTM formation in αCaMKII^{T286A} mice. Moreover, in normal wild-type mice MIS generation appears suppressed due to signaling induced by αCaMKII autophosphorylation.

Intriguingly, the identification of MIS generation as a LTM mechanism further underlines the importance of LTP as a LTM mechanism, as the generation of MIS can be considered as a kind of 'morphological LTP'. However, in contrast to 'chemical LTP' this 'morphological LTP' may be less modifiable as a multitude of molecular processes would have to be involved versus simple AMPA receptor endocytosis in the case of 'chemical LTP'. Thus, the question arises whether LTM, which involves MIS generation, has the same properties as LTM in wild-type mice. To this end, retrieval-induced modulation of contextual fear LTM in αCaMKII^{T286A} mice was studied (Radwanska et al., 2011). Retrieval can induce a destabilization process that is compensated by a protein synthesis-dependent reconsolidation process (Nader & Einarsson, 2010). These retrieval-induced processes are thought to be important for memory maintenance and memory updating. Memory destabilization can be assessed by pharmacologically blocking protein synthesis-dependent reconsolidation so that retrieval only induces destabilization that should erase the memory. However, if retrieval does not lead to memory erasure when reconsolidation is blocked, then the mechanisms of memory destabilization are impaired. Destabilization of contextual fear LTM after retrieval was found to be impaired in αCaMKII^{T286A} mice in contrast to normal wild-type mice. Whilst this indicates that T286 autophosphorylation is required for retrieval-induced LTM destabilization, it leaves the possibility that MIS generation forms a LTM that is less modifiable than LTM that does not involve MIS.

4. Conclusion and outstanding questions

The αCaMKII^{T286A} mice have been a great tool for identification of a novel LTM mechanism in the absence of NMDA receptor-dependent LTP at CA1 synapses. Studies with these mutants suggest that MIS generation is sufficient for LTM formation when LTP is absent. MIS generation requires multiple, massed

Please cite this article in press as: Giese, K. P., et al. Generation of multi-innervated dendritic spines as a novel mechanism of long-term memory formation. *Neurobiology of Learning and Memory* (2015), http://dx.doi.org/10.1016/j.nlm.2015.04.009

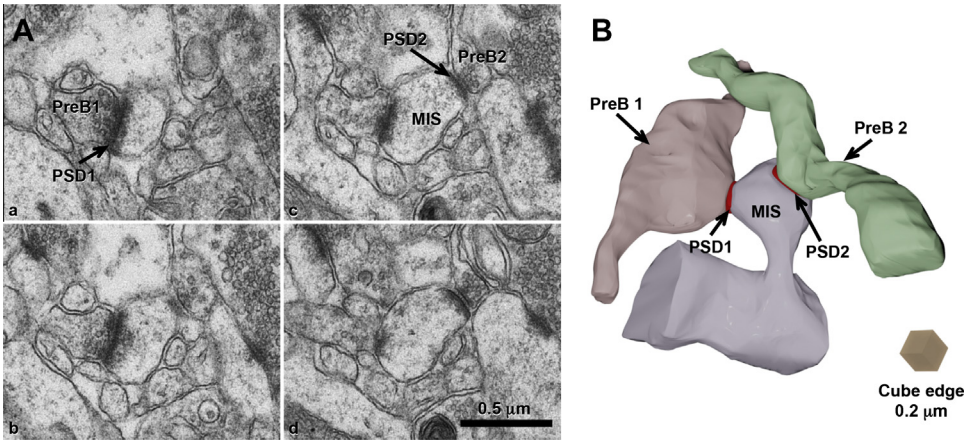

Fig. 1. Example of a multi-innervated dendritic spine (MIS) in hippocampal area CA1 of wild-type mice. A. Serial electron microscopy images of a MIS making contacts with two presynaptic boutons. B. 3D reconstruction of the MIS from the series shown in panel A. PreB, presynaptic bouton; PSD, postsynaptic density.

training, as a single training trial is not sufficient. MIS generation was found after contextual fear conditioning of αCaMKII^{T286A} mice (Radwanska et al., 2011). It is expected that MIS generation also enables LTM formation after training in other hippocampus-dependent memory tasks such as passive avoidance (see, Irvine et al., 2005). However, training of αCaMKII^{T286A} mice in the Morris water maze may not lead to MIS generation in the hippocampus, because even after very intensive training (12 trials per day) the mutants cannot form a spatial memory.

Based on our contextual fear conditioning experiments, LTM that involves MIS generation might not be destabilized upon retrieval impairing LTM updating. The signaling pathways underlying the crosstalk between LTP and MIS generation are illustrated in Fig. 2. In wild-type mice after NMDA receptor activation T286 autophosphorylation of αCaMKII prolongs signaling at the synapse inducing a signal to the nucleus to activate expression of plasticity-related proteins that are required for late LTP (Redondo & Morris, 2011). Further, T286 autophosphorylation signaling suppresses MIS generation by preventing mTOR-dependent translation of psd95 mRNA. However, in the absence of T286 autophosphorylation signaling mTOR signaling leads to increased PSD-95 expression at the synapse. PSD-95 binds to nNOS to produce a retrograde signal to attract a presynaptic terminal(s). Moreover, increased PSD-95 expression can lead to formation of a separate PSD in the same spine, which can allocate AMPA and NMDA receptors in the adequate postsynaptic position to establish a new synaptic contact.

The hypothesis that MIS generation is a LTM mechanism after multiple training trials raises questions for further investigation, such as: (1) Is MIS generation associated with impaired LTM formation and updating in normal, wild-type animals under conditions when LTP is compromised? For example, normal ageing is associated with impaired NMDA receptor-dependent LTP at CA1 synapses (Bach et al., 1999; Murphy et al., 2004). Thus, is age-associated LTM impairment caused by MIS generation? (2) Does abnormal MIS generation cause deficits in LTM formation

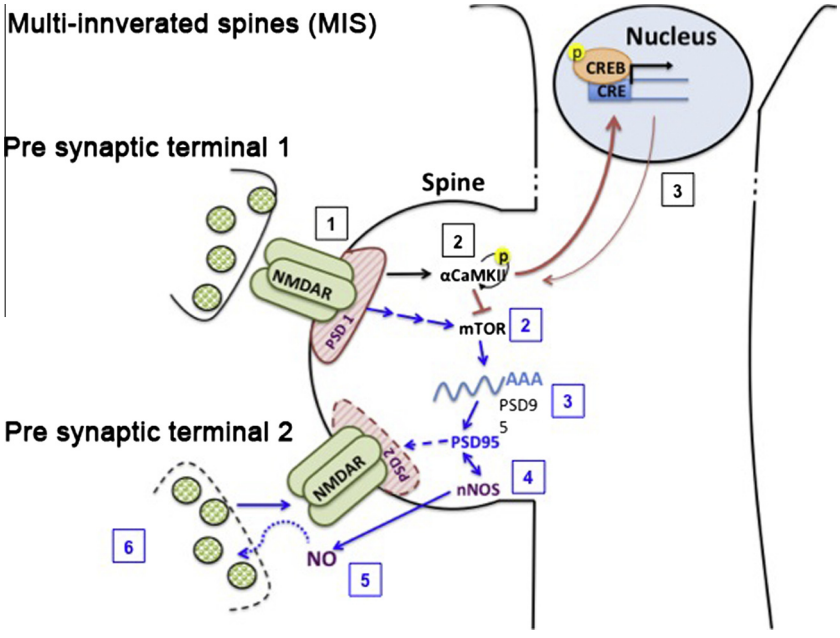

Fig. 2. Signaling mechanisms showing crosstalk between LTP induction and MIS generation. Normally, NMDA receptor activation leads to T286 autophosphorylation of αCaMKII that induces synthesis of plasticity-related proteins for late LTP and additionally blocks activation of mTOR signaling (1–3). Without T286 autophosphorylation multiple NMDA receptor activations can induce mTOR signaling to locally synthesize PSD-95 protein. PSD-95 binds to nNOS to produce the retrograde messenger NO to attract a presynaptic terminal onto the existing dendritic spine to generate a MIS (2–6).

Please cite this article in press as: Giese, K. P., et al. Generation of multi-innervated dendritic spines as a novel mechanism of long-term memory formation. *Neurobiology of Learning and Memory* (2015), http://dx.doi.org/10.1016/j.nlm.2015.04.009

and updating in diseases that impair LTP, such as the early stages of Alzheimer's disease (Sheng, Sabatini, & Sudhof, 2012)? (3) Is the suppression of MIS generation dismissed in models of autism, so that next to LTP MIS are generated and cause deficits in LTM formation?

Acknowledgments

We thank Stephanie Rothe for help with preparing Fig. 2. This work has been funded by the MRC and BBSRC.

References

Bach, M. E., Barad, M., Son, H., Zhuo, M., Lu, Y. F., Shih, R., et al. (1999). Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proceedings of the National Academy of Sciences of the United States of America, 96*, 5280–5285.

Bridi, M. S., & Abel, T. (2013). The NR4A nuclear receptors mediate transcription-dependent hippocampal synaptic plasticity. *Neurobiology of Learning and Memory, 105*, 151–158.

Cooke, S. F., Wu, J., Plattner, F., Errington, M., Rowan, M., Peters, M., et al. (2006). Autophosphorylation of alphaCaMKII is not a general requirement for NMDA receptor-dependent LTP in the adult mouse. *Journal of Physiology, 574*, 805–818.

Fiala, J. C., Feinberg, M., Popov, V., & Harris, K. M. (1998). Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. *Journal of Neuroscience, 18*, 8900–8911.

Giese, K. P. (2012). Long-term potentiation and memory. In K. P. Giese (Ed.), *Memory mechanisms in health and disease* (pp. 1–17). Singapore: World Scientific Publishing.

Giese, K. P., Fedorov, N. B., Filipkowski, R. K., & Silva, A. J. (1998). Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science, 279*, 870–873.

Gruart, A., Munoz, M. D., & Delgado-Garcia, J. M. (2006). Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice. *Journal of Neuroscience, 26*, 1077–1087.

Holt, C. E., & Schuman, E. (2013). The central dogma decentralized: New perspectives on RNA function and local translation in neurons. *Neuron, 80*, 648–657.

Irvine, E. E., Danhiez, A., Radwanska, K., Nassim, C., Lucchesi, W., Godaux, E., et al. (2011). Properties of contextual memory formed in the absence of alphaCaMKII autophosphorylation. *Molecular Brain, 4*, 8.

Irvine, E. E., Vernon, J., & Giese, K. P. (2005). AlphaCaMKII autophosphorylation contributes to rapid learning but is not necessary for memory. *Nature Neuroscience, 8*, 411–412.

Jones, M. W., Errington, M. L., French, P. J., Fine, A., Bliss, T. V., Garel, S., et al. (2001). A requirement for the immediate-early gene zif268 in the expression of late LTP and long-term memories. *Nature Neuroscience, 4*, 289–296.

Kennedy, M. B. (2000). Signal-processing machines at the postsynaptic density. *Science, 290*, 750–754.

Lee, S. J., Escobedo-Lozoya, Y., Szatmari, E. M., & Yasuda, R. (2009). Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature, 458*, 299–304.

Martin, S. J., Grimwood, P. D., & Morris, R. G. (2000). Synaptic plasticity and memory: An evaluation of the hypothesis. *Annual Review of Neuroscience, 23*, 649–711.

Murphy, G. G., Fedorov, N. B., Giese, K. P., Ohno, M., Friedman, E., Chen, R., et al. (2004). Increased neuronal excitability, synaptic plasticity, and learning in aged Kvbeta1.1 knockout mice. *Current Biology, 14*, 1907–1915.

Nabavi, S., Fox, R., Proulx, C. D., Lin, J. Y., Tsien, R. Y., & Malinow, R. (2014). Engineering a memory with LTD and LTP. *Nature, 511*, 348–352.

Nader, K., & Einarsson, E. O. (2010). Memory reconsolidation: An update. *Annals of the New York Academy of Sciences, 1191*, 27–41.

Nikonenko, I., Boda, B., Steen, S., Knott, G., Welker, E., & Muller, D. (2008). PSD-95 promotes synaptogenesis and multiinnervated spine formation through nitric oxide signaling. *Journal of Cell Biology, 183*, 1115–1127.

Nikonenko, I., Jourdain, P., & Muller, D. (2003). Presynaptic remodeling contributes to activity-dependent synaptogenesis. *Journal of Neuroscience, 23*, 8498–8505.

Petrak, L. J., Harris, K. M., & Kirov, S. A. (2005). Synaptogenesis on mature hippocampal dendrites occurs via filopodia and immature spines during blocked synaptic transmission. *Journal of Comparative Neurology, 484*, 183–190.

Radwanska, K., Medvedev, N. I., Pereira, G. S., Engmann, O., Thiede, N., Moraes, M. F., et al. (2011). Mechanism for long-term memory formation when synaptic strengthening is impaired. *Proceedings of the National Academy of Sciences of the United States of America, 108*, 18471–18475.

Redondo, R. L., & Morris, R. G. (2011). Making memories last: The synaptic tagging and capture hypothesis. *Nature Reviews Neuroscience, 12*, 17–30.

Restivo, L., Vetere, G., Bontempi, B., & Ammassari-Teule, M. (2009). The formation of recent and remote memory is associated with time-dependent formation of dendritic spines in the hippocampus and anterior cingulate cortex. *Journal of Neuroscience, 29*, 8206–8214.

Sheng, M., Sabatini, B. L., & Sudhof, T. C. (2012). Synapses and Alzheimer's disease. *Cold Spring Harbor Perspectives in Biology, 4*.

Silva, A. J. (2003). Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. *Journal of Neurobiology, 54*, 224–237.

Villers, A., Giese, K. P., & Ris, L. (2014). Long-term potentiation can be induced in the CA1 region of hippocampus in the absence of alphaCaMKII T286-autophosphorylation. *Learning & Memory, 21*, 616–626.

Whitlock, J. R., Heynen, A. J., Shuler, M. G., & Bear, M. F. (2006). Learning induces long-term potentiation in the hippocampus. *Science, 313*, 1093–1097.

Yasuda, H., Barth, A. L., Stellwagen, D., & Malenka, R. C. (2003). A developmental switch in the signaling cascades for LTP induction. *Nature Neuroscience, 6*, 15–16.