Experience-Dependent, Rapid Structural Changes in Hippocampal Pyramidal Cell Spines

Morphological changes in dendritic spines may contribute to the fine tuning of neural network connectivity. The relationship between spine morphology and experience-dependent neuronal activity, however, is largely unknown. In the present study, we combined 2 histological analyses to examine this relationship: 1) Measurement of spines of neurons whose morphology was visualized in brain sections of mice expressing membrane-targeted green fluorescent protein (Thy1-mGFP mice) and 2) Categorization of CA1 neurons by immunohistochemical monitoring of Arc expression as a putative marker of recent neuronal activity. After mice were exposed to a novel, enriched environment for 60 min, neurons that expressed Arc had fewer small spines and more large spines than Arc-negative cells. These differences were not observed when the exploration time was shortened to 15 min. This net-balanced structural change is consistent with both synapse-specific enhancement and suppression. These results provide the first evidence of rapid morphological changes in spines that were preferential to a subset of neurons in association with an animal’s experiences.

Keywords: behavior, hippocampus, immediate-early gene, plasticity, sparse coding, spine dynamics

Introduction

Dendritic spines, tiny protrusions that form the postsynaptic sites of most excitatory synapses (Harris and Stevens 1989), are the basic functional units of neuronal integration. Dendritic spine size positively correlates with the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) current before and after the induction of long-term synaptic plasticity in hippocampal slices (Matsuzaki et al. 2001, 2004), and electrical stimulation that is classically used to induce long-term potentiation and depression leads to spine formation and retraction, respectively (Nagerl et al. 2004). Theoretical studies have suggested that the formation and elimination of spines together constitute a potential mechanism for memory (Stepanyants et al. 2002). Findings from experiments in in vivo models support the notion that the structural plasticity of spines is linked to memory-associated circuit reorganization (Moser et al. 1997; Geinisman et al. 2001). For example, the density of distinct spines in the hippocampus increases 24 h after eye-blink conditioning (Leuner et al. 2003), and in vivo imaging of spines in the whisker barrel model suggests that the change in somatotopic representation induced by whisker-trimming is associated with stabilization of a subset of new spines over a period of days (Holtmaat et al. 2006). Such rapid structural changes linked to natural neuronal activity during behavior have not been described.

To analyze the effects of experience-evoked activity on spine morphology, we combined 2 histological techniques: 1) neuronal structure was visualized in brain sections of mice expressing membrane-targeted green fluorescent protein (Thy1-mGFP mice) (Richards et al. 2005); 2) a subset of neurons potentially activated in mice during brief exposure to a novel, enriched environment was detected by monitoring protein expression of the immediate-early gene Arc/Arg3.1 (Link et al. 1995; Lyford et al. 1995) by immunohistochemistry. Although the direct demonstration of an association between Arc signals and cellular activity is still lacking, accumulating evidence suggests that neuronal activity of cells precedes the Arc expression (Lyford et al. 1995; Steward and Worley 2001; Shepherd et al. 2006). Furthermore, the selectivity of the Arc-positive cell population for a particular environment (Guzowskii et al. 1999; Ramirez-Amaya et al. 2005) and the inhibition of Arc expression following memory-impairing fornix lesions (Fletcher et al. 2006) suggest that Arc-expressing neurons are involved in neural encoding and memory formation.

Accordingly, we compared the spine morphology of Arc-expressing and nonexpressing neurons to examine how brief exposure to a novel, enriched environment alters the spine structure in hippocampal CA1 pyramidal cells.

Materials and Methods

Novel, Enriched Environment Exposure Procedures

Experiments were performed according to the guide for the care and use of laboratory animals of the University of Tokyo. Male Thy1-mGFP mice (line 21, gift from Drs V. de Paola and P. Caroni; De Paola et al. 2003) which express membrane-targeted green fluorescent protein (mGFP) in a small number of CA1 neurons, were housed 2–4 littermates per cage in a vivarium with controlled temperature and humidity (23 ± 1 °C, 50 ± 10%) and free access to food and water on a 12-h light/dark cycle. All mice were handled daily for 5 days and were not exposed to a novel environment for at least 7 days before the mice were exposed to the novel, enriched environment at 8–11 weeks of age. Half of the Thy1-mGFP mice were placed in a plastic cage (37D × 21W × 15h cm, Fig. S1A) that was larger than their home cage (HC) in a novel room for 15 min (N15) or 60 min (N60), whereas their age-matched littersmates remained in their home cages (HC group). There were 2 sets of HC mice, one for each of the N15 and N60 groups. Five novel objects and 4 small unfamiliar food pellets were placed in the cage in which 4 distinct markings were displayed on the walls. No apparent eating behavior was observed in the novel cage. N15 mice were retained in their HC since 45 min after exposure to the environment. For the experiment described in Figure S3, mice were injected with saline or scopolamine hydrobromide (2 mg/kg, i.p., Wako, Osaka, Japan) 20 min prior to exposure to the environment for 60 min. Immediately after the 60-min session, all mice...
were anesthetized by inhalation of diethyl ether and perfused transcardially with chilled phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). The dissected brains were postfixed for at least 2 h at 4°C, immersed first in 20% and then in 30% sucrose in 0.1 M PB (+4°C, >72 h in total), frozen, and coronally sectioned (ca. Bregma -1.3 to -2.0 mm) at a thickness of 40 μm. Pair-wise brain sections of mice from the HC and N15 (or N60) groups were mounted on the glass slides and processed for immunohistochemistry in the same solutions.

**Immunohistochemistry Procedures**

Slide-mounted sections were incubated in 0.2% Triton X-100 for 30 min and treated in 1% H2O2, diluted in PBS for 15 min. After blocking with 2% normal goat serum for 1 h, the slides were incubated in anti-Arc antibody (rabbit, 1:8000; Lyford et al. 1995) for 48 h at 4°C, followed by anti-rabbit biotinylated secondary antibody (1:500, Vector Laboratories, Burlingame, CA), sometimes in combination with NeuroTrace-135/455 blue-fluorescent Nissl stain (1:50, Molecular Probes, Eugene, OR), for 60 min at room temperature. Immunolabeling was amplified by incubating with avidin-biotin complex (1:100, Vector Laboratories) for 60 min. The staining was visualized using the Cy-3 TSA fluorescent system (1:20, PerkinElmer Life Sciences, Boston, MA). All binding procedures were followed by 3 PBS washes.

**Confocal Microscopy**

Images of the hippocampal CA1 region were captured with a confocal microscope (MRC-1000, Zeiss, Oberkochen, Germany) equipped with 488-nm argon and 543 helium/neon lasers. First, to classify mGFP-positive pyramidal cells as Arc(+)/Arc(-), image stacks (1.0 μm thickness × 21 planes) of Arc and mouse Gad1 from the pyramidal cell layer were collected using a 60× objective (NA 1.2, water immersion). Laser power and gain parameters for Arc images were set such that pixel intensities were not saturated and were kept constant for all sections on the same slide. Image stacks (0.5 μm × 21 planes) of basal dendrites of mGFP-positive pyramidal cells were then collected with 3×digital zoom (0.067 μm/pixel). To capture triple-colored images including Nissl staining, a Zeiss LSM510 confocal microscope equipped with a blue diode laser and a 20× objective (NA 0.5) was used.

**Image Analyses**

Morphological parameters (length, head size, and spine density per unit length of dendrite) of spines in basal dendrites that were included in the images except on the top and bottom planes of the stacks were measured. Raw mGFP images (Fig. S1B) were processed by median filtration and deconvolution (MetaMorph, Molecular Devices, Downingtown, PA). This processing approach produced smaller standard deviation values (raw, 81 ± 47; processed, 49 ± 25; P < 0.05 by Student’s t-test). Representative image of an mGFP (green)-expressing pyramidal cell, Arc immunoreactive cells (red), and Nissl stain (blue) in an N60 mouse. The inset shows spines (arrowheads) on a dendrite of an mGFP(+) cell. SO, stratum oriens where basal dendrites extend; SP, stratum pyramidale; SR, stratum radiatum. Scale bars, 20 μm in (A) and (D), 1 μm in the inset of (D).
Results

Arc Expression after Exposure to the Novel, Enriched Environment

Thy1-mGFP mice were exposed to a novel, enriched environment for 15 min (N15), 60 min (N60) or kept in their HCs. Immediately after the 60-min session, the brains were collected. Approximately 25% of the hippocampal CA1 neurons in the N15 and N60 brains were Arc(+), whereas only 3% of the CA1 neurons in HC samples were Arc(+) (Fig. 1A). The proportion of cells that was Arc(+) as well as the intensity of Arc immunoreactivity was similar between the N15 and N60 groups (Fig. 1B,C). In the N60 group, administration of the muscarinic receptor antagonist scopolamine, which impairs the formation of hippocampal-dependent spatial memory (Buresova et al. 1986), before placing the mice in the environment decreased the proportion of Arc(+) cells to 1% (Fig. S3). This finding supports a possible link between Arc expression and memory formation, and suggests that the Arc expression was not merely due to mental and physical stress or other physical differences between HC and N15/N60.

Time-Dependent Reduction in the Number of Small Spines in Arc(+) Cells

Morphological analysis of mGFP-labeled spines on the basal dendrites of CA1 pyramidal cells (Fig. 1D, see also Fig. S1B for additional images of dendritic segments) revealed that overall spine density in N15 (mean ± SEM: 1.47 ± 0.05/μm dendrite) and N60 (1.26 ± 0.06/μm dendrite) cells was similar to that of the respective littermate HC control group cells (1.38 ± 0.06 and 1.26 ± 0.07/μm dendrite, respectively; both P’s > 0.05 by Student’s t-test), suggesting that the total number of spines was not altered by the environmental exposure. Segregation of Arc(+) and Arc(-) cells in the same samples, however, revealed that the spine density of Arc(+) cells was lower than that of Arc(-) cells in the N60 group (Fig. 2C), but not in the N15 group (Fig. 2A,B). The difference in the N60 group was also confirmed by comparing the averaged data from individual animals (Arc(-), 1.29 ± 0.08; Arc(+), 1.06 ± 0.07/μm dendrite; n = 5 animals, P < 0.01 by paired t-test). The difference in the spine density became more evident if only spines with a head size of less than 0.5 μm in diameter were considered (Fig. 2D-F). Namely, Arc(+) cells in the N60 group had lower small spine density compared with Arc(-) cells in both HC and N60 group (Fig. 2D). This difference was more evident in dendrites 30-50 μm from soma (Fig. S4A,B,D). Furthermore, we prepared Figure S4A,C to assure a concern on the location-related bias, because the somata of Arc(+) cells were preferentially localized nearer to stratum oriens in the cell layer in both N60 and N15 groups, although the underlying mechanism is not known. The figure shows that the Arc(+) cells had fewer small spines, regardless of the location of the soma.

Increase in Large-Head Spines in Arc(+) Neurons

Arc(+) and Arc(-) neurons also exhibited differences in large-head spines. We analyzed 2 pooled datasets of spines from the 3 groups, 1) Arc(-) cells in HC (there were no mGFP-labeled, Arc(+) cells in HC), 2) Arc(-) cells in either N15 or N60, and 3) Arc(+) cells in either N15 or N60 as 2 datasets for each condition (N15/N60). We first defined large-head spines as spines whose size was among the largest 5% of all measured spines in the each of the pooled datasets and evaluated the size distribution among the 3 groups. In the dataset including N15 mice, allocation of large-head spines in each group was close to 33%, which is chance level (Fig. 3A). In the dataset including N60 mice, however, large-head spines were more frequently found on Arc(+) cells (Fig. 3D). This divergence was robust, even if the definition of a large-head spine was expanded to the largest 25% (Fig. 3B,E). To examine whether this distribution was within possible stochastic fluctuations, we created surrogate data by randomly shuffling the rank order of spine size in the each pooled dataset. A significant deviation from the random data was rarely found in the N15 dataset (Fig. 3C), HC versus N15 Arc(-), HC versus N15 Arc(+), and HC versus N60 Arc(-) (Fig. S5A-C), but many more large spines were present in Arc(+) cells in the N60 mice (Fig. 3F and Fig. S5D) than expected by stochastic fluctuation.

The reason that significant difference was not detected in average density of large spines in Figure 2D may be due to the large deviations among density from cells within the Arc(+) group (Chen et al. 2007). To show the information, we plotted parameters of each cell, and actually found that very-large spine density of Arc(+) cells varied greatly in the N60 group (Fig. 3G-I). In addition, we found that the spine size for the N15 and N60 group resulted in a significant inverse correlation between the densities of small (<0.5 μm) and very-large (>0.8 μm, approximately correspond to the largest 5% of all spines) spines in the N60 group (Fig. 3HI); the HC control group showed no statistically significant correlation (Fig. 3G).

The time course of these shifts in spine size frequency might be related to changes in neuronal networks demonstrated by the mouse’s behavior. In separate groups of mice re-exposed to the same environment the next day, the locomotor activity of mice in the N60 group was significantly lower than that of the N15 group, suggesting recognition memory of the previous day’s experience in the N60 mice (Fig. S6).

It was confirmed that mGFP expression did not interfere with Arc detection or the intensity and pattern of Arc expression (Fig. 1 and Fig. S7D,E). Furthermore, Thy1-mGFP transgenic mice did not differ in the locomotor activity compared with wild-type mice, suggesting that the mGFP expression also did not affect the behavior (Fig. S7A-C).

Discussion

Dendritic Spine Changes in Living Animals

The novel finding of the present study was that rapid structural changes in hippocampal spines were induced by exposure to a novel, enriched environment. There were no clear differences in the spines between the Arc(-) and Arc(+) cells in the N15 group, thereby excluding the possibility that only neurons that already had spines with a different morphology preferentially expressed Arc during exploratory behavior. Although it is possible that spine morphology was also changed within the 15 min of exposure to the novel environment in the N15 group and that this effect was reversed during the subsequent 45-min period in the HC, the fact remains that the changes persisted between the Arc(-) and Arc(+) cells in the N60 group. These data together suggest that the spine changes occurred as a result of the duration of the exposure to a novel, enriched environment. That is, the reduction in the number of small spines is likely due to spine elimination or shrinkage during exposure.
exploration of the environment, whereas the increase in the number of large spines likely reflects enlargement of existing spines and/or de novo emergence of large spines.

Relationship between Behavior and Spine Changes
The structural differences between N15 and N60 seemed to be related with mouse behavior in the re-exposure session (Fig. S6). Significantly suppressed exploratory behavior in N60 on day 2 suggests that the extent and/or quality (Levins and Besheer 2006) of familiarization during the exposure to the environment on day 1 was greater in the N60 than N15, and that spine reorganization may underlie memory formation in the behaving animals.

Rapid Spine Changes in a Subset of Neurons
The present findings indicated that relatively rapid (but not immediate, <60 min) structural changes occurred in hippocampal pyramidal cell spines. Various behavioral paradigms such as eye-blink conditioning, exposure to an enriched environment, and chronic stress induce structural reorganization of spines that has been observed from 1 day to several months later (Rampon et al. 2000; Leuner et al. 2003; Silva-Gomez et al. 2003; Mitra et al. 2005), whereas in in vitro experiments, bidirectional spine plasticity has been described within 1 h of stimulation (Engert and Bonhoeffer 1999; Matsuzaki et al. 2004; Zhou et al. 2004). This is the first report, however, of the detection of rapid spine reorganization after stimulation of living animals with short exposure to a stimulus.

Figure 2. Effects of exposure to a novel, enriched environment on spine density. Spine density per micron of dendrite length and distributions of spine head sizes in N15 (A, B) and N60 (C, D) cells are shown. Arc(+) cells in the N60 group possessed fewer small spines compared with Arc(−) cells in both the N60 and HC groups, whereas there was not a statistically significant difference in the N15 group. Arc(+) cells in the N60 group possessed fewer small spines compared with Arc(−) cells in both the N60 and HC groups, whereas there was not a statistically significant difference in the N15 group. (A, B) HC, n = 19 (1081 spines) from 4 mice; Arc(−), n = 23 (1513 spines); Arc(+) n = 10 cells (564 spines) from 4 mice. (C, D) HC, n = 16 cells (851 spines) from 6 mice; Arc(−), n = 19 cells (1283 spines); Arc(+) n = 8 cells (350 spines) from 8 mice. Error bars indicate standard error of the mean. *P < 0.05 by Tukey’s post hoc test in (A, C). **P < 0.01/3, *P < 0.05/2 by Bonferroni–Holm test after repeated-measures 2-way ANOVA in (B, D). These significant differences were reproduced in another independent experiment. (E) There was a pronounced decrease in spines smaller than 0.5 μm on Arc(+) cells in the N60 group (red), but not in the N15 (gray) group. (F) Spine density ratio between the Arc(+) and Arc(−) cells for the small (<0.5 μm) and large (>0.5 μm) spines. Note the distinctly opposite patterns for small and large spines in the N60 group.
We were able to detect rapid spine changes by dividing the cells into Arc (+) and Arc(−) groups to isolate those cells that had recently been active. Thus, even when it is difficult to detect the structural changes in spines averaged across the entire cell population, this method allowed us to detect clear rapid changes of spines in a specific subset of neurons that were activated by the stimulus to the animals.

Possible Effects by Reduction of Small Spines after Exploring Activity

The depressed densities of small (<0.5 \( \mu m \)) spines imply changes in functional neuronal circuits. Although silent synapses would be included in these decreased ones, some spines should form functional synapses (Harris and Stevens 1989; Noguchi et al. 2005). Thus, the decrease of spines would mean some form of depression in synaptic transmission at the time. Further, it might lead to long-term depression accompanied by spine shrinkage and retraction (Nagerl et al. 2004; Zhou et al. 2004).

Another important point is that smaller spines have greater potentiality to undergo long-term potentiation (Matsuzaki et al. 2004). Assuming that some of Arc (+) neurons commit to convey specific information thereafter, the reduction of small spines might contribute to the functional differentiation.

Enlargement of Spines in Limited Number

It is notable that exposure to the novel, enriched environment in our experiment enlarged only a limited number of spines.
These findings are similar to those of a recent study in which the authors estimated the number of spines with detectable transport of newly synthesized glutamate receptors (GluR1), implying enhanced transmission, during fear conditioning; only ~3% of all spines in Fos-positive neurons had preferential transport of newly synthesized GluR1 (Matsuzaki et al. 2008). Our results, however, are not consistent with the ~30% increases in the density of distinct spines observed 24 h after eye-blink conditioning (Leuner et al. 2003). The difference might reflect the difference in the strength and quality of stimulations to the brain region. Future studies to evaluate the strength of the relationship between learning or neuronal network stimulation and spine reorganization are necessary. We speculate that although only a minor proportion of spines would be enlarged, the more substantial inputs they would create would be critically important for competitive neural circuit reactivation. Previous findings that a larger spine evokes a larger EPSP (Matsuzaki et al. 2001) and that spatiotemporally clustered large inputs can be supra-linearly summed due to the initiation of a dendritic spike (Losonczy and Magee 2006) support this notion.

Conclusions

The present study provides the first evidence of rapid, coordinated spine enlargement and spine elimination in neurons activated by an animal’s exposure to a novel environment, and provides an estimate of the extent of structural synaptic changes that occur during a natural animal experience. The ability to monitor structural changes in activated and nonactivated populations of neurons provides an important new and simple paradigm for studying the molecular and synaptic mechanisms of natural structural reorganization.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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Notes

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Address correspondence to email: makiky-tky@umin.ac.jp.

References

Bevins RA, Besheer J. 2006. Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study ‘recognition memory’. Nat Protoc. 1:1306–1311.

Bloodgood BL, Sabatini BL. 2005. Neuronal activity regulates diffusion across the neck of dendritic spines. Science. 310:866–869.

Buresova O, Bolhuis JJ, Bures J. 1986. Differential effects of cholinergic blockade on performance of rats in the water tank navigation task and in a radial water maze. Behav Neurosci. 100:476–482.

Chen J, Kitanishi T, Ikeda T, Matsuki N, Yamada MK. 2007. Contextual learning induces an increase in the number of hippocampal CA1 neurons expressing high levels of BDNF. Neurobiol. Learn. Mem. 88:409–415.

De Paola V, Arber S, Caroni P. 2003. AMPA receptors regulate dynamic equilibrium of presynaptic terminals in mature hippocampal networks. Nat Neurosci. 6:491–500.

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

Fletcher BR, Calhoun ME, Rapp PR, Shapiro ML. 2006. ForinI lesions decouple the induction of hippocampal arc transcription from behavior but not plasticity. J Neurosci. 26:1507–1515.

Gehrman Y, Berry RW, Disterhoft JF, Power JM, Van der Zee EA. 2001. Associative learning elicits the formation of multiple-synapse boutons. J Neurosci. 21:5568–5573.

Guzowski JF, McNaughton BL, Barnes CA, Worley PF. 1999. Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. Nat Neurosci. 2:1120–1124.

Harris KM, Stevens JK. 1989. Dendritic spines of CA1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. J Neurosci. 9:2982–2997.

Holtmaat A, Wilbrecht L, Knott GW, Welker E, Svoboda K. 2006. Experience-dependent and cell-type-specific spine growth in the neocortex. Nature. 441:979–983.

Holtmaat AJ, Trachtenberg JT, Wilbrecht L, Shepherd GM, Zhang X, Knott GW, Svoboda K. 2005. Transient and persistent dendritic spines in the neocortex in vivo. Neuron. 45:279–291.

Leuner B, Falduto J, Shors TJ. 2003. Associative memory formation increases the observation of dendritic spines in the hippocampus. J Neurosci. 23:659–665.

Link W, Konietzko U, Kauselmann G, Krug M, Schwannek B, Frey U, Kuhl D. 1995. Somatodendritic expression of an immediate early gene is regulated by synaptic activity. Proc Natl Acad Sci U S A. 92:5734–5738.

Losonczy A, Magee JC. 2006. Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. Neuron. 50:291–307.

Lyford GL, Yamagata K, Kaufmann WE, Barnes CA, Sanders LK, Copeland NG, Gilbert DJ, Jenkins NA, Lanahan AA, Worley PF. 1995. Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. Neuron. 14:433–445.

Matsuzaki M, Honkura N, Ellis-Davies GCR, Kasai H. 2004. Structural basis of long-term potentiation in single dendritic spines. Nature. 429:761–766.

Mitra R, Jadhav S, McEwen BS, Vyas A, Chattarji S. 2005. Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala. Proc Natl Acad Sci U S A. 102:9371–9376.

Moser MB, Tronnecd M, Egelnd T, Andersen P. 1997. Spatial training in a complex environment and isolation alter the spine distribution differently in rat CA1 pyramidal cells. J Comp Neurol. 380:373–381.

Nagerl UV, Eberhorn N, Cambridge SB, Bonhoeffer T. 2004. Bidirectional activity-dependent morphological plasticity in hippocampal neurons. Neuron. 44:759–767.

Noguchi J, Matsuzaki M, Ellis-Davies GC, Kasai H. 2005. Spine-neck geometry determines NMDA receptor-dependent Ca2+ signaling in dendrites. Neuron. 46(4):609–622.

Ramirez-Amaya V, Vazdarjanova A, Mikhael D, Rosi S, Worley PF, Barnes CA. 2005. Spatial exploration-induced Arc mRNA and protein expression: evidence for selective, network-specific reactivation. J Neurosci. 25:1761–1768.

Ramon C, Tang YP, Goodhouse J, Shimizu E, Kyin M, Tsien JZ. 2000. Enrichment induces structural changes and recovery from non-spatial memory deficits in CA1 NMDAR1-knockout mice. Nat Neurosci. 3:238–244.
Richards DA, Mateos JM, Hugel S, de Paola V, Caroni P, Gabwiler BH, McKinney RA. 2005. Glutamate induces the rapid formation of spine head protrusions in hippocampal slice cultures. Proc Natl Acad Sci U S A. 102:6166-6171.

Shepherd JD, Rumbaugh G, Wu J, Chowdhury S, Plath N, Kuhl D, Huganir RL, Worley PF. 2006. Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. Neuron. 52:475-484.

Silva-Gomez AB, Rojas D, Juarez I, Flores G. 2003. Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. Brain Res. 983:128-136.

Stepanyants A, Hof PR, Chklovskii DB. 2002. Geometry and structural plasticity of synaptic connectivity. Neuron. 34:275-288.

Steward O, Worley PF. 2001. Selective targeting of newly synthesized Arc mRNA to active synapses requires NMDA receptor activation. Neuron. 30:227-240.

Zhou Q, Homma KJ, Poo MM. 2004. Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron. 44:749-757.