FK506-Binding Protein 12.6/1b, a Negative Regulator of \([\text{Ca}^{2+}]\), Rescues Memory and Restores Genomic Regulation in the Hippocampus of Aging Rats

John C. Gant, * Eric M. Blalock, * Kuey-Chu Chen, Inga Kadish, Olivier Thibault, Nada M. Porter, and Philip W. Landfield

1Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, Kentucky 40536, and 2Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, Alabama 35294

Hippocampal overexpression of FK506-binding protein 12.6/1b (FKBP1b), a negative regulator of ryanodine receptor \([\text{Ca}^{2+}]\) release, reverses aging-induced memory impairment and neuronal \([\text{Ca}^{2+}]\) dysregulation. Here, we tested the hypothesis that FKBP1b also can protect downstream transcriptional networks from aging-induced dysregulation. We gave hippocampal microinjections of FKBP1b-expressing viral vector to male rats at either 13 months of age (long-term, LT) or 19 months of age (short-term, ST) and tested memory performance in the Morris water maze at 21 months of age. Aged rats treated ST or LT with FKBP1b substantially outperformed age-matched vector controls and performed similarly to each other and young controls (YCs). Transcriptional profiling in the same animals identified 2342 genes with hippocampal expression that was upregulated/downregulated in aged controls (ACs) compared with YCs (the aging effect). Of these aging-dependent genes, 876 (37%) also showed altered expression in aged FKBP1b-treated rats compared with ACs, with FKBP1b restoring expression of essentially all such genes (872/876, 99.5%) in the direction opposite the aging effect and closer to levels in YCs. This inverse relationship between the aging and FKBP1b effects suggests that the aging effects arise from FKBP1b deficiency. Functional category analysis revealed that genes downregulated with aging and restored by FKBP1b were associated predominantly with diverse brain structure categories, including cytoskeleton, membrane channels, and extracellular region. Conversely, genes upregulated with aging but not restored by FKBP1b associated primarily with glial–neuroinflammatory, ribosomal, and lysosomal categories. Immunohistochemistry confirmed aging-induced rarefaction and FKBP1b-mediated restoration of neuronal microtubular structure. Therefore, a previously unrecognized genomic network modulating diverse brain structural processes is dysregulated by aging and restored by FKBP1b overexpression.

Key words: aging; calcium; cytoskeleton; FKBP12.6; microarray; ryanodine receptor

Introduction

Dysregulation of neuronal \([\text{Ca}^{2+}]\) concentrations and of \([\text{Ca}^{2+}]\)-dependent physiologic responses is among the most consistent neurobiological manifestations of mammalian brain aging (Landfield and Pitler, 1984; Michaelis et al., 1984; Gibson and Peterson, 1987; Landfield, 1987; Khachatruian, 1989; Reynolds and Carlen, 1989; Thompson et al., 1996; Verkhratsky and Toescu, 1998; Heimond and Jaffe, 2005; Murchison and Griffith, 2007; Thibault et al., 2007).
2007; Oh et al., 2010). Further, Ca\(^{2+}\) dysregulation has been associated with aging-related cognitive dysfunction in multiple species (Disterhoft et al., 1996; Thibault and Landfield, 1996; Tombaugh et al., 2005; Murphy et al., 2006; Luebke and Amatrudo, 2012; Gant et al., 2015) and evidence of Ca\(^{2+}\) dysregulation also is present in postmortem Alzheimer’s disease (AD) brains and in mouse models of AD (Nixon et al., 1994; Gibson et al., 1996; Stuttmann et al., 2006; Kuchibhotla et al., 2008; Overk and Masliah, 2017).

Little is known about the mechanisms underlying aging-related Ca\(^{2+}\) dysregulation, although a strong candidate mechanism, disruption of FK506-binding protein 12.6/1b (FKBP1b), has emerged recently. FKBP1b is a member of the FKBP family of immunophilins (Kang et al., 2008) and in muscle cells is an established negative regulator of intracellular Ca\(^{2+}\) release from ryanodine receptors (RyRs) (Zalk et al., 2007; Lehnart et al., 2008; MacMillan and McCarron, 2009).

Recently, we found that FKBP1b negatively regulates Ca\(^{2+}\) release from RyRs in brain neurons as well and, additionally, inhibits Ca\(^{2+}\) influx via membrane L-type Ca\(^{2+}\) channels (Gant et al., 2011, 2014, 2015). Selective knock-down of FKBP1b in the hippocampus of young rats recapitulates the Ca\(^{2+}\) dysregulation aging phenotype of enlarged RyR-dependent Ca\(^{2+}\) potentials and currents (Gant et al., 2011). Further, chronic-stress-induced FKBP1b disruption is associated with cognitive dysfunction (Liu et al., 2012). In addition, short term (ST) virally mediated overexpression of FKBP1b in rat hippocampus reverses aging-related elevation of Ca\(^{2+}\) transients and spatial memory deficits (Gant et al., 2015). Moreover, hippocampal FKBP1b protein and gene expression declines with normal aging in rats (Kadish et al., 2009; Gant et al., 2015) and in early-stage AD (Blalock et al., 2004). These findings suggest that FKBP1b is a key regulator of neuronal Ca\(^{2+}\) homeostasis and cognitive processing that is disrupted during aging.

Transcriptional and translational processes, notably for the activity-regulated, cytoskeletal-associated protein (Arc), also have been linked to memory, synaptic growth, and dendritic remodeling (Steward et al., 1998; Guzowski et al., 2000; Schafe and LeDoux, 2000; Ploski et al., 2008; Lee and Silva, 2009; Alberini and Kandel, 2014; Fletcher et al., 2014), but it is unclear whether they are modulated by the FKBP1b network. Electrical activity at synapses and plasmalemmal membranes can trigger genomic responses via multiple Ca\(^{2+}\) signaling cascades or Ca\(^{2+}\)-dependent transcription factors (Graef et al., 1999; Gall et al., 2003; Greer and Greenberg, 2008) and elevated intracellular Ca\(^{2+}\) concentrations induce electrophysiological and structural signs of deterioration (Scharfman and Schwartzkroin, 1989; Bezprozvonny and Mattson, 2008) that activate genomic pathways involved in cell death. In addition, FKBP1b and its protein isoform, FKBP1a, regulate non-RyR-dependent pathways, including the mechanistic target of rapamycin (mTOR) pathway, which modulates brain-derived neurotrophic factor (BDNF) and other transcriptionally active factors (Binder and Scharfman, 2004; Hoefler et al., 2008; Lynch et al., 2008). These multiple pathways of potential downstream regulation make it important to determine whether and how the Ca\(^{2+}\) regulator FKBP1b interacts with plasticity-associated transcriptional processes.

Here, we used a multidisciplinary approach to test the hypothesis that FKBP1b overexpression also counters selective aging-related alterations in transcription. In addition, to determine whether long-term (LT) FKBP1b overexpression is safe and efficacious and may be a candidate preventive therapy, we compared ST hippocampal FKBP1b overexpression with LT FKBP1b overexpression, which is initiated in midlife when memory impairment first begins to emerge (Forster and Lal, 1992; Gallagher and Rapp, 1997; Markowska, 1999; Wyss et al., 2000; Bizon et al., 2009; Scheinert et al., 2015). Together, the results indicate that LT and ST virally mediated FKBP1b overexpression can prevent and reverse, respectively, important aspects of aging-related brain decline.
30 s. On day 5 (reference memory probe), the platform was removed and the subjects were started in the quadrant opposite the goal quadrant and allowed to swim for 60 s.

On day 8, the platform was placed in a new quadrant location. The subjects were then given 3 trials, 1 from each nongoal quadrant, to learn the new platform location (reversal training). On day 9 (reversal memory probe), the platform was removed and subjects were allowed to swim for 60 s. On day 10, visual acuity and locomotor ability were assessed with the platform made visible by raising it 1 cm above the water surface and hanging a bright white contrasting marker 6 inches above the platform location. The subjects were again given three trials from each of the three nongoal quadrants to find the platform.

Three days after the visual acuity task, the brains were harvested for qRT-PCR, gene chip, and immunohistochemistry studies as in prior work (Kadish et al., 2009; Seary et al., 2012; Latimer et al., 2014; Gant et al., 2015). Animals were anesthetized with pentobarbital (Fatal Plus, 50 mg/kg, i.p.). After perfusion with 150 ml of cold 0.9% saline, the brains were removed and hemisected. For qPCR and gene chip studies, the dorsal hippocampus was removed from one hemisphere. This tissue was placed in RNase-free sample tubes and stored at −80°C until further use. For immunohistochemistry studies, the other hemisphere was post-fixed overnight in 4% paraformaldehyde, cryoprotected by submersion in 15% sucrose/PBS solution, and then placed in antifreeze/30% sucrose solution for storage until sectioning.

Immunohistochemistry (IHC) methods were similar to those we have described previously (Kadish and Van Groen, 2003; Gant et al., 2015). Coronal sections (30 µm) were cut on a freezing sliding microtome. The following primary antibodies were used for overnight incubation: rabbit anti-FKBP12.6 (1:500, sc-98742; Santa Cruz Biotechnology) and mouse Microtubule Associated Protein-2 (MAP2, 1:500, MAB3418; Millipore).

After incubation, sections were rinsed and transferred to the solution containing appropriate biotinylated secondary antibody for 2 h and then rinsed and transferred to the solution containing ExtrAvidin for 2 h. Sections were then incubated for 3 min with Ni-enhanced DAB solution. To obtain similarly stained material, sections from all animals were stained simultaneously in the same staining tray. The immunostained sections of the dorsal hippocampus were digitized using an Olympus DP73 camera and the resulting images were analyzed using ImageJ. For optical densitometric analysis of MAP2 immunohistochemistry, two sections of the apical dendritic layer (stratum radiatum) of hippocampal CA1 pyramidal neurons were measured per animal. Investigators were blinded to animal number and condition and all photomicrographs were taken with the same settings of the DP73 camera. The staining pattern of the anti-FKBP 12.6/1b antibody was highly similar to that seen in two prior studies of hippocampal FKBP1b with this antibody, one in which we selectively knocked down FKBP1b with short hairpin RNA targeting FKBPA (Gant et al., 2006) and one in which we overexpressed FKB1b and also compared endogenous FKBP1b in young versus ACs (Gant et al., 2015). In both prior studies, the topography of FKBP1b immunostaining was highly similar to that in the present study and the antibody clearly detected the experimental manipulations and the aging difference in FKBP1b expression in CA1. In addition, the vendor validates antibody specificity by Western blot analyses and we performed negative control studies by omitting the primary antibody and using only secondary in adjacent brain sections from the same subjects. These negative controls produced no staining in our rat brain tissues. Dorsal hippocampal RNA was extracted according to standard protocols and evaluated using an Agilent Technologies Bioanalyzer. All samples were of sufficient quality and did not differ significantly among treatment groups (RNA integrity number: 9.4 ± 0.1 for all groups; p = 0.16, ANOVA across the YC, AC, ST, and LT groups). Extracted RNA was used for both PCR and microarray measures. For RT-PCR mRNA quantification, one-step real-time qRT-PCR was used. qRT-PCR amplification was performed as described previously (Gant et al., 2015) using an ABI Prism 7700 sequence detection system (Applied Biosystems) and a RNA-to-CT 1-step TaqMan kit (Life Technologies). All samples were run in duplicate in a final volume of 30 µl containing 25–50 ng of cellular RNA and a Taqman Fam–MGB probe (Rn00575368_m1; Life Technologies) with an amplicon spanning a 116 bp rat FKBP1b cDNA region.

Cycling parameters for all assays were as follows: 30 min at 48°C, 10 min at 95°C followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C. The RNA levels of Gapdh were used as normalization controls for RNA quantification.

Microarray procedures were similar to those in our prior microarray studies on hippocampal aging in rats (Blalock et al., 2003; Rowe et al., 2007; Kadish et al., 2009). Briefly, RNA extracted from dorsal hippocampus was labeled and hybridized to Affymetrix Rat Gene 1.0 ST arrays (one array per animal). Gene signal intensities for microarrays were calculated using the Robust Multiarray Average algorithm (Bolstad et al., 2003) at the transcript level and data were associated with vendor-provided annotation information. For experimental design and statistical analyses, see Fig. 1. To test the hypothesis that expression in selective genomic systems parallels the changes in memory performance induced by aging and, in the opposite direction, by FKBP1b rescue, we compared YC rats and AC rats (that received bilateral injections of AAV-eGFP) with aged rats that received bilateral dorsal hippocampal injections of AAV-FKBP1b. The FKBP1b-expressing virus was microinjected into aging rats either 2 months (ST) or 8 months (LT) before testing spatial reference and reversal learning in the MWM. All three aged groups were tested together at 21 months of age along with young (5-month-old) controls (Fig. 1). The reference memory task tests the ability to learn an aspect of the task that remains constant, whereas reversal learning comprises elements of both working memory and executive cognitive function (Webster et al., 2014) and is well recognized to be particularly susceptible to impairment with aging (Bartus et al., 1979; Stephens et al., 1985). Initial statistical assessments of behavioral comparisons used ANOVA. If significance was found at the ANOVA level (α = 0.05), then protected Fisher’s least significant difference (pLSD) pairwise contrasts (α = 0.05) between groups were also performed.

Microarray profiling was undertaken with the goal of identifying the FKBP1b-sensitive transcriptome. The n’s chosen for microarray studies were based on our prior work (Blalock et al., 2003; Rowe et al., 2007; Kadish et al., 2009; Blalock et al., 2010), which found that n’s of 5–10 per group are sufficient to identify a distinct hippocampal aging transcriptome consistently. For transcriptional profiling in the present study, a subgroup of six subjects was selected from each of the four parent treatment groups (N = 24 rats, one array per rat). To reduce false negatives and to maximize the transcriptional responses to elevated FKBP1b expression, we selected subjects for the ST and LT subgroups that showed FKBP1b expression levels above the medians for the parent groups. The YC and AC groups exhibited low within-group variance of FKBP1b ex-
expression (see Fig. 3) and these subgroups were selected at random from within their parent groups.

The Rat Gene Array used for the microarray analysis contains 29,218 total probe sets, which we filtered before statistical testing to retain only the 14,828 probe sets characterized by unique gene annotations and by signal intensity (expression) adequate to show presence of the gene (defined as unlogged signal intensity ≥40 on ≥4 arrays in the study). Expression of each of these 14,828 genes was tested by one-way ANOVA to identify genes exhibiting significant differences in expression across the four groups. Outlier values (>2 SDs of the group mean) were treated as missing values. Transcriptional profiling analyses can involve thousands of statistical comparisons and thus require assessment of multiple testing error. We estimated the error contributed by multiple testing using the approach was used to test the Gene Ontology (GO) database (biological process, cellular component, molecular function) for categories overrepresented by genes of each template pattern. Medium classification stringency was applied. Functional annotation clustering output from DAVID was transferred to flat files and the most significant annotation from within each cluster was identified. Among these, unique functional annotations with significant Ease Scores are reported along with the number of associated pathway genes.

In a further assessment of possible contributions of chance to these large-scale analyses, the numbers of genes that were identified as belonging to a particular pattern were compared with the numbers of genes that would be expected to fall within that pattern by chance. The probability that a gene would fall into a particular pattern by chance was estimated by applying a Monte Carlo analysis in which the same statistical procedures (ANOVA test, post hoc pLSD, and template pattern) and statistical

**Figure 2.** Both LT and ST FKBP1b overexpression countered age-related decline in spatial memory. **A,** Reference memory probe. FKBP1b treatments countered age-related deficits in reference memory probe performance. **B,** Reversal memory probe. FKBP1b treatments countered age-related deficits in the reversal memory probe trial. **C,** Cued testing. With visual cues prominently highlighting location of the escape platform, no group differences were found, indicating that memory test results were not due to differences in locomotor and/or visual abilities. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001 significant pairwise contrast versus AC.

**Figure 3.** Hippocampal FKBP1b mRNA and protein levels were increased substantially by LT AAV-FKBP1b overexpression. Top, qRT-PCR quantification of hippocampal FKBP1b mRNA expression (FKBP1b/Gapdh) for each treatment group (one-way ANOVA on ranks, p = 0.000050; for pairwise contrast vs AC, *p = 0.05; ***p = 0.0001). Bottom, Immunostaining for hippocampal FKBP1b expression. Representative photomicrographs are shown from YC (A), AC (B), aged ST FKBP1b (C), and aged LT FKBP1b (D). Note the substantial increase in FKBP1b expression at both the mRNA and protein levels, particularly in the LT-FKBP1b group. sp, Stratum pyramidale; DG, dentate gyrus. Scale bar, 500 μm.
criteria \((\alpha = 0.05)\) were used, but applied to random numbers rather than actual gene signal intensity values. The Monte Carlo procedure was run 1000 times, with a newly generated set of random numbers in each iteration. The average number of genes found to fall within each expression pattern across 1000 iterations of random data was then used as an estimate of the number of genes expected to fall within that pattern by chance. The binomial test \((\alpha = 0.05)\) was used to determine whether the number actually found in the pattern exceeded significantly the number expected by chance.

**Results**

*FKBP1b* overexpression improved spatial reference and reversal memory for both LT and ST aged groups (Fig. 2)

A total of 52 rats in four groups completed our spatial memory testing protocol in the MWM (10 YC, 13 AC, 13 ST, and 16 LT rats). For the 3 groups of aged AAV-treated rats, behavioral testing began at 21 months of age, either 8 (LT) or 2 (ST) months after AAV injection. Training in the MWM reference memory task was performed over 4 d, with 3 training trials per day. Over the 4 d, all groups showed acquisition of the task as indicated by a distinct decline in latency and path-length to find the platform \((F_{(3,48)} = 11.58, p = 1.86e-10; F_{(11,528)} = 7.41, p = 7.20e-12,\) respectively, repeated-measures ANOVA). Although there was a strong trend for young animals to outperform ACs during training, no significant effects of treatment were seen in latency and path length measures over this 4 d training phase, similar to results in Gant et al. (2015).

On the fifth day of the task, the platform was removed and recall of the platform location was probed with a single retention trial (Fig. 2A; reference memory probe). There was a main effect of treatment group for both latency \((F_{(11,528)} = 11.58, p = 1.86e-10, F_{(3,48)} = 7.41, p = 7.20e-12,\) ANOVA). As reported in multiple studies, the AC group exhibited significantly longer path lengths and higher latencies to find the platform compared with YC rats. In contrast, neither *FKBP1b*-treated aged group differed from YCs and both showed significantly reduced latency compared with ACs (vs ACs: LT, \(p = 0.05, ST, p = 0.05; YCs, p = 0.002, pLSD\)). The path length to platform results were highly similar to latency, although the differences between the *FKBP1b* groups and the ACs were only of borderline significance (vs ACs: LT, \(p = 0.078, ST, p = 0.08, YCs, p = 0.006, pLSD\)).

Training on the reversal memory task was conducted on the eighth day after 2 d of rest. The location of the platform was changed and the rats were given 3 trials in 1 d to learn the new location. Over the three reversal training trials, there were no significant differences in latency or path length among any of the groups, nor was there significant improvement (data not shown). On the ninth day, the platform was again removed and rats were tested for their retention of the new platform location on a single retention trial (Fig. 2B, reversal memory probe). There were substantial main effects of treatment on latency to platform \((F_{(3,48)} = 9.57, p = 0.000046, ANOVA)\) and path length to platform location \((F_{(3,48)} = 7.97, p = 0.0002, ANOVA)\). AC animals again exhibited highly significant deficits in path length and latency compared with YCs, but both ST and LT groups exhibited path length and latency scores that were highly similar to those of YCs and significantly reduced compared with AC animals ( latency; AC vs: LT, \(p < 0.0001, ST, p < 0.0001; YC, p < 0.0001;\) path length, AC vs: LT, \(p = 0.0001, ST, p = 0.0001; YC, p = 0.0008, pLSD; Fig. 2B)\). There was also a main effect of treatment group for platform crossings during the reversal retention test \((F_{(3,48)} = 7.37, p = 0.0004; ANOVA)\), with the YC, ST, and LT groups showing significantly greater platform crossings compared with ACs (ACs vs: YCs, \(p < 0.0001; ST; p < 0.025; LT, p < 0.01, pLSD\)).

On the 10th day of the protocol, a cued retention test was given with visual cues highlighting the platform’s location. All groups found the platform rapidly and no significant group differences were present in latency, path length, or swim speed in locating the platform (Fig. 2C, cued trial), indicating that aging-related changes in locomotor and visual acuity did not account for the differences in memory performance.

The ST and LT groups were statistically indistinguishable from each other on all latency and path length measures in the behavioral testing protocols. These results suggest that the reversal by ST and prevention by LT of aging-dependent memory impairment may be mediated by similar cellular mechanisms despite the differences in the duration of exposure.
Table 1. Functional categories overrepresented by genes assigned to the four expression templates reflecting aging ± FKBP1b sensitivity

| I. Downregulated with age, unchanged by FKBP1b | n   | p-value | II. Downregulated with age, upregulated by FKBP1b | n   | p-value |
|-----------------------------------------------|-----|---------|-----------------------------------------------|-----|---------|
| Serine hydroxide activity                     | 18  | 0.0019  | Cytoskeleton                                   | 39  | 9.6E-04 |
| Cellular amino acid derivative biosynthesis   | 9   | 0.0029  | Passive transmembrane transporter activity      | 19  | 0.0039  |
| Integral to plasma membrane                   | 27  | 0.0050  | Extracellular region                            | 41  | 0.0080  |
| Amine metabolic process                       | 27  | 0.0082  | Ectodermal development                          | 8   | 0.0249  |
| Tissue morphogenesis                          | 19  | 0.0150  | Regulation of actin cytoskeleton                | 6   | 0.0265  |
| Positive regulation of gene expression        | 36  | 0.0341  | Metalloendopeptidase activity                   | 6   | 0.0335  |
|                                               |     |         | Regulation of MAP kinase activity                | 8   | 0.0387  |

III. Upregulated with age, unchanged by FKBP1b | n   | p-value | IV. Upregulated with age, downregulated by FKBP1b | n   | p-value |
| Translational elongation                      | 36  | 5.9E-23 | Extracellular matrix                            | 20  | 1.0E-04 |
| Lysosome                                      | 35  | 2.7E-13 | Primary transmemb. Transporter activity         | 11  | 9.1E-04 |
| Immune system process                         | 76  | 5.0E-11 | Regulation of cell motion                       | 15  | 0.0010  |
| Endosomal part                                | 12  | 3.6E-06 | Basalpleral plasma membrane                     | 15  | 0.0021  |
| Immune effector process                       | 21  | 3.7E-06 | Golgi cisterna                                  | 6   | 0.0027  |
| Ribosomal small subunit biogenesis            | 7   | 2.0E-05 | Blood vessel development                        | 16  | 0.0035  |
| Leukocyte activity during immune response     | 11  | 2.3E-05 | Membrane-bounded vesicle                        | 29  | 0.0076  |
| Antigen processing and presentation           | 15  | 4.0E-05 | Response to oxygen levels                       | 13  | 0.0162  |
| Response to stress                            | 97  | 7.3E-05 | Response to estrogen stimulus                   | 10  | 0.0246  |
| T cell activation                             | 15  | 0.0010 | Membrane organization                           | 16  | 0.0247  |
| Regulation of acute inflammatory response     | 8   | 0.0020 | Intracellular transport                         | 23  | 0.0281  |
| Protein kinase cascade                        | 26  | 0.0036 | Organelle membrane                              | 39  | 0.0385  |
| Carbohydrate binding                          | 25  | 0.0045 | Homeostasis of number of cells                  | 8   | 0.0419  |
| Protein amino acid phosphorylation            | 44  | 0.0056 |                                                |     |         |
| Response to oxidative stress                  | 18  | 0.0095 |                                                |     |         |
| Response to lipid                             | 7   | 0.0134 |                                                |     |         |
| Cell-cell adhesion                            | 17  | 0.0324 |                                                |     |         |

Shown are gene ontology category annotations overrepresented by genes exhibiting expression patterns matching one of the four templates (Fig. 4) in direction of change and sensitivity to FKBP1b listed in order of significance: Left: Aging effect unchanged by FKBP1b. Right: Aging effect countered (opposite direction change) by FKBP1b. (n, number significant genes in category; p-value, modified Fisher’s exact test/EASE score). Also see Table 1–1 (available at https://doi.org/10.1523/JNEUROSCL.2234-17.2017.E1-1).

AAV-FKBP1b injection increased hippocampal FKBP1b protein and gene expression, particularly in the LT group (Fig. 3)

Because the Affymetrix Rat Gene 1.0 ST microarray used in the present study does not include the probe set for FKBP1b, we used qRT-PCR to evaluate the effectiveness of AAV-FKBP1b injection for inducing FKBP1b expression in hippocampus. FKBP1b/Gapdh expression is plotted as a function of treatment group in Figure 3. There was a highly significant increase in FKBP1b expression (Fstat = 18.449, p = 0.000050, ANOVA on ranks, Kruskal–Wallis). By pairwise contrast (Fisher’s LSD on ranks), the increased expression was significant in ST (p = 0.012) and highly significant in LT (p < 0.001). IHC in representative animals indicated that hippocampal FKBP1b protein upregulation paralleled FKBP1b mRNA increases in AAV-FKBP1b-treated rats and was particularly intense in LT animals (Fig. 3, bottom).

In contrast to our prior findings (Blalock et al., 2004; Kadish et al., 2009; Gant et al., 2015), we did not observe differences in endogenous FKBP1b expression between the AC and YC groups (Fig. 3, top). This may be due to differences between studies in tissue dissection. In the present study, we collected tissue from whole dorsal hippocampus, whereas in prior work, we measured expression primarily in the CA1 region (Blalock et al., 2004; Kadish et al., 2009; Gant et al., 2015). Therefore, the aging effect on FKBP1b in CA1 might have been obscured in the present work because of dilution from less age-sensitive hippocampal regions. Further studies will be needed to fully elucidate the topographic distribution of aging changes in FKBP1b protein and gene expression, as well as the role of potential functional changes (Lehnart et al., 2008).

Transcriptional profiling (Fig. 4)

To identify genes with expression that paralleled aging and FKBP1b’s cognitive effects in the same animals, we first distin-
between LT and ST effects across all 876 FKBP1b-sensitive genes. A highly significant proportion of FKBP1b-sensitive genes (822/876; 93.8%) were changed in the same direction by both ST and LT (p ≤ 1E-12, binomial test). Further, the effect sizes of ST and LT treatment genes (expressed as log2-fold change vs AC) were strongly correlated (r = 0.85, p = 1.3E-24; Pearson’s test, data not shown). These results indicate that ST and LT FKBP1b treatments influenced gene expression similarly. The ST and LT groups also performed nearly identically on all behavioral measures. Based on these similarities and because the genome ontology functional category analysis (see below) provides greater statistical confidence in overrepresentation of a given category with increasing numbers of genes assigned to that functional category, we combined the ST and LT lists of FKBP1b-sensitive genes into a single list, such that a gene was considered an FKBP1b-sensitive gene if it differed from ACs with ST and/or LT treatment.

Remarkably, only four of the 876 aging-dependent and FKBP1b-sensitive genes (Eif3g, Pla2g7, S100b, and Snapc2) exhibited exacerbation of aging effects by FKBP1b, whereas the other 872 changed in opposite directions with aging and FKBP1b treatment. Accordingly, the anomalous four genes were excluded from functional category analyses and the remaining aging-dependent genes (2338) were parsed into one of four gene expression templates (Fig. 4, right, templates I–IV), which reflected direction of a gene’s expression change with aging (up or down) and whether the gene did (Fig. 4, templates II and IV), or did not (Fig. 4, templates I and III) show the counteracting effect of FKBP1b treatment (LT or ST vs AC; p ≤ 0.05, Fisher’s post hoc LSD).

To determine whether the number of genes identified in each template was greater than expected by chance, we ran a Monte Carlo simulation using the same statistical analysis and template assignment strategy, but with randomly generated numbers substituted for signal intensity values (see Materials and Methods). The numbers of genes actually observed for each reported pattern exceeded by >11-fold the number expected by chance based on the simulation (Fig. 4; = 0.00001, binomial test for each pattern), indicating a strong biological effect.

GO functional categories associated with genes matching each of the four template patterns (Fig. 4) of aging- and FKBP1b-sensitive genes (Table 1). Lists of genes assigned to each of the four expression templates described above (Fig. 4) were uploaded separately to DAVID (Huang da et al., 2007) and the biological process, cellular component, and molecular function gene ontologies were interrogated. In each significant cluster, the most statistically overrepresented functional category by DAVID analysis with more than three genes was retained. The retained functional categories for each of the four templates are shown in Table 1 (for individual groups with each identified functional pathway, see Table 1-1, available at https://doi.org/10.1523/JNEUROSCI.2254-17.2017.1-1).

Genes downregulated with aging and unchanged by FKBP1b (AC downregulated vs YC, template I) overrepresented GO annotations associated with neurotransmitter metabolism and biosynthesis in neurons. Their downregulation with aging may reflect a reduction of neuronal growth and synthetic processes.

Genes upregulated with aging and unchanged by FKBP1b (AC upregulated vs YC, template III) very strongly overrepresented functional categories associated with upregulated translational elongation, lysosome activity, and immune signaling, which appear to reflect major turnover of proteins, cholesterol transport, and new biosynthesis of apolipoproteins by astrocytes, as well as activated neuroinflammatory signaling by microglia. Similar patterns have been seen in multiple studies (Weindruch and Prolla, 2002; Blalock et al., 2003; Rowe et al., 2007; Kadish et al., 2009; VanGuilder et al., 2011; Chen et al., 2013). Surprisingly, inflammatory responses have generally not been found to correlate closely with learning or memory (Rowe et al., 2007; Kadish et al., 2009; VanGuilder et al., 2011) and their resistance to counteraction by FKBP1b, seen here, extends those prior findings.

Genes downregulated with aging and upregulated by FKBP1b (AC downregulated vs YC, FKBP1b upregulated vs AC, template II) strongly overrepresented GO categories related to intracellular and extracellular structure, including: cytoskeleton, extracellular region passive transmembrane transporter activity, ectoderm development, regulation of actin cytoskeleton organization, and regulation of MAP kinase activity (Table 1), as considered further in Discussion.

Genes upregulated with aging and downregulated by FKBP1b (AC upregulated vs YC, FKBP1b downregulated vs AC, template IV) overrepresented GO category annotations related to extracellular remodeling and transporter activity, including extracellular matrix, primary transmembrane transporter activity, blood vessel development, regulation of cell motion, and membrane-bounded vesicle (Table 1), potentially reflecting extracellular reorganization, glial and endothelial cell cytokinesis, and transport of substances between activated cells.

MAP2: downregulation with aging and restoration by FKBP1b (Fig. 5, Table 2). The “cytoskeleton” category was the most statistically significant downregulated with aging and upregulated by FKBP1b functional category and has been consistently identified as being age dependent in our prior studies on hippocampal gene expression associations with memory (Blalock et al., 2003; Rowe et al., 2007; Kadish et al., 2009). Accordingly, to confirm that these gene expression alterations were reflected in cytoskeletal changes at the protein level, we used semiquantitative IHC to analyze MAP2, an abundant somatodendritic MAP that reflects general microtubular structure (Fig. 5). MAP2 protein expression was significantly reduced (F1,10 = 7.792, p = 0.0015, ANOVA, *post hoc pLSD p ≤ 0.001) with age and rescued by LT treatment (pLSD p = 0.01) and possibly ST treatments (borderline significant, pLSD p = 0.06), consistent with the view that the cytoskeletal genomic alterations are reflected in overall function and protein structure of the cytoskeleton.

Possible downstream mediators of FKBP1b effects Calpain-1, calcineurin, and other Ca2+-related genes (Table 3). Because FKBP1b is a negative regulator of hippocampal neuronal Ca2+-transients (Gant et al., 2011, 2015), it seemed feasible that pathological Ca2+-related signaling during aging might be restored to young levels by FKBP1b overexpression. We therefore identified all Ca2+-related genes on the microarray and determined which showed age-related changes in gene expression that were significantly counteracted by FKBP1b treatment (i.e., met the criteria for template II or IV in Fig. 4). Although Ca2+-related genes as a category did not show significant overrepresentation of genes with aging changes that were countered by FKBP1b, the genes for two Ca2+-sensitive enzymes frequently associated with brain aging, neuronal plasticity, and AD, Cnp1 (calpain-1) and Ppp3cc (calcineurin, Nixon et al., 1994; Rozkalne et al., 2011; Furman and Norris, 2014), as well as a Ca2+ release-activated Ca2+ channel (ORA11) were altered by aging and restored by FKBP1b treatment (Table 3). Therefore, these Ca2+-related genes may be sensitive downstream mediators of some aspects of both
age-dependent neuronal Ca\(^{2+}\) dyshomeostasis and restoration of regulation by overexpression of FKBP1b.

mTOR pathway genes

Genes in the mTOR pathway were also of specific interest because this pathway is inhibited by the immunosuppressant drug rapamycin via complex formation with FKBP1b/1a. Moreover, FKBP1a, a close isoform of FKBP1b, negatively regulates mTOR in the brain, even in the absence of rapamycin (Hoeffer et al., 2008). These interactions of FKBP1s and mTOR appear to occur independently of FKBP1b/1a-dependent regulation of Ca\(^{2+}\) release from RyRs. Therefore, to determine whether mTOR pathway expression is altered by FKBP1b activity, we investigated mTOR pathway gene expression relative to FKBP1b overexpression. Twenty-four mTOR pathway genes were identified by searching the GO database and the literature (Johnson et al., 2013). Of these 24, only two, Hif1α and Nfkb1, were significantly altered with both age (upregulated) and FKBP1b (downregulated), showing that the mTOR pathway was not statistically overrepresented by FKBP1b-sensitive genes (n.s., \(p = 0.78\), binomial test). Protein–protein interactions importantly regulate mTOR signaling. Nevertheless, the lack of change in mTOR pathway gene expression suggests that FKBP1b’s genomic effects were not mediated by the mTOR pathway.

### Discussion

The present studies provide the first evidence that FKBP1b, a negative regulator of intracellular [Ca\(^{2+}\)], modulates a previously unrecognized genomic network regulating structural organization of the brain. In the hippocampus, this network appears to be specifically targeted and dysregulated by aging. However, as shown here, FKBP1b overexpression can largely reverse or prevent aging-dependent alterations in gene expression in the network. In this study, FKBP1b overexpression restored regulation of this network in parallel with cognitive rescue in the same aged rats. Therefore, this genomic evidence adds strong new support for the hypothesis that FKBP1b is a linchpin of neuronal homeostasis that functions at multiple levels, including regulation of Ca\(^{2+}\), maintenance of structural integrity, and preservation of cognitive function.

### Aging-induced changes in gene expression

As in our prior work, we found a high proportion of hippocampal genes (2342/14,928) that were altered significantly with aging (Fig. 4, the “aging effect”). Further, the expression levels of 37% of these aging-altered genes also differed significantly between AC and aged FKBP1b rats (876/2342 genes, the “FKBP1b effect”). Regardless of whether the aging effect for a given gene was upregulation or downregulation, FKBP1b shifted the expression levels for essentially all of these genes (872 of 876, 99.5%) back toward their YC levels. This remarkably consistent restorative action of FKBP1b strongly suggests that disruption of FKBP1b or closely associated molecules may underlie a significant portion of the aging effect for the 872 FKBP1b-regulated genes.

Nevertheless, deficient FKBP1b function does not account for all aspects of genomic change with brain aging. Of the aging-altered genes, a majority (1462/2342, ~63%) were not affected by FKBP1b overexpression. Notably, those associated with upregulated glial-inflammatory processes were unaffected by FKBP1b (Table 1). Therefore, it appears that other aging processes (e.g., glial) also modulate hippocampal transcription, but operate independently from or upstream of the FKBP1b-sensitive genomic network.

### FKBP1b overexpression reveals an apparent genomic network that regulates neuronal structure and is targeted by aging

FKBP1b-restored genes were predominantly associated with GO functional categories related to diverse components of brain structure. In particular, “cytoskeleton,” “passive membrane transport” (ion channel proteins that add structure to lipid bilayer membranes), and “extracellular region” were the three functional categories most overrepresented by genes with expression that declined with aging and increased with FKBP1b (Table 1). The “cytoskeleton” category was represented by numerous genes related to actin, intermediate filaments, and microtubule assembly, whereas the extracellular region category was represented by multiple genes encoding collagens and matrix metalloproteinases, two families essential for extracellular matrix assembly/remodeling. Similarly, among genes with expression that increased with aging and declined with FKBP1b, the most overrepresented category was “extracellular matrix,” which, despite also focusing on extracellular space, was represented by a markedly different group of genes. The “extracellular matrix” category included genes encoding proteoglycans, growth factors, and other proteins associated with glial activation and blood vessel development (Table 1 and Table 1-1, available at https://doi.org/10.1523/JNEUROSCI.2234-17.2017.11-1). These patterns of expression in FKBP1b-sensitive aging genes appear to reflect an aging-related shift in biosynthetic...
activity and differentiation from neuronal and extracellular structure to glial processes/compartments. The disparate structural systems altered by aging and restored by FKBP12.6 suggest the operation of a novel genomic network that coordinates structural assembly among diverse brain components. This network is particularly targeted by aging, but the disruptive effects of aging can, as shown here, be prevented or reversed by FKBP12.6 overexpression in neurons.
It seems of considerable interest that cytoskeletal remodeling in dendritic spines has been shown to play a critical role in LTP (Lynch and Baudry, 1987; Lynch et al., 2008; Briz and Baudry, 2016). In the present study, cytoskeletal gene downregulation with aging and protection by FKBP1b was among the major effects and was confirmed at the protein level by IHC analyses of MAP2, the primary MAP of somatodendritic compartments (Fig. 5). However, more than one FKBP1b-associated process likely participates in memory formation. Accordingly, defining the precise pathways linking FKBP1b overexpression to counteraction of age-related memory decline will require substantial further study.

The gene encoding calpain-1, a major Ca\(^{2+}\)-activated, cytoskeletal-degrading protease, was upregulated with aging and downregulated by FKBP1b (Table 3). This is the inverse pattern of that for cytoskeletal genes and suggests that calpain-1 upregulation might mediate important aspects of downregulation and degradation of the cytoskeleton during aging. Conversely, by downregulating calpain-1 expression, FKBP1b overexpression may restore or maintain neuronal structural integrity.

**Efficacy and safety of ST and ST effects of FKBP1b overexpression**

Another goal of the present study was to test whether LT FKBP1b overexpression in middle-aged animals could prevent the emergence of cognitive impairment as effectively as ST FKBP1b can reverse it (Gant et al., 2015). Results showed clearly that FKBP1b overexpression did not lose behavioral efficacy over extended time because both the LT and ST FKBP1b treatment groups substantially outperformed ACs and performed similarly to each other and to young animals (Fig. 2). These results show that cognitive impairment can be prevented by LT treatment beginning in middle-aged animals and reversed by ST treatment in aged animals.

An interesting question not addressed here is whether animals already performing at a high baseline, including young animals and the small percentage of aged animals that are unimpaired (Bäckman et al., 1996; Gallagher and Rapp, 1997; Tombaugh et al., 2002; Rowe et al., 2003, 2007; Curlik et al., 2014; Ménard et al., 2015), show improved memory with FKBP1b. It will be important in future studies to determine whether unimpaired animals are resistant or sensitive to the actions of FKBP1b on memory.

Relative to safety issues, FKBP1b expression was significantly higher in LT compared with ST rats (Fig. 3), indicating increased expression over the 9-month exposure or that expression is greater when initiated in middle-aged versus aged animals. Regardless, the health of LT rats appeared comparable to that of ST or age-matched vector control rats because there was only one death and no apparent morbidity among LT rats over the 9-month course of the experiment. The clinical literature also indicates that AAV-mediated gene overexpression is generally long lasting and safe in brain tissue, with an extremely low probability of inducing oncogenic or other deleterious changes in DNA (Kapli et al., 2007; McCown, 2010).

**Do normal-aging-induced cytoskeletal conditions model neurofibrillary tangle pathology of AD?**

Advanced age is the leading risk factor for AD (Reitz et al., 2011), suggesting that some processes that drive normal brain aging also increase susceptibility to AD. Considerable evidence indicates that Ca\(^{2+}\) dysregulation is present in both normal brain aging and AD (see Introduction) and therefore is positioned to play a role in age-dependent susceptibility to AD. Cytoskeletal dysfunction, in the form of neurofibrillary tangles is one of the hallmarks of AD pathology. It is correlated with synaptic degeneration and characterized by microtubule rarefaction and hyperphosphorylation of the MAP tau (Wisniewski and Terry, 1973; Grundke-Iqbal et al., 1979; Masliah et al., 1991; Jicha et al., 1997; Serrano-Pozo et al., 2011; Webster et al., 2014). Here, we found that hippocampal cytoskeletal gene expression is downregulated with aging and, in parallel with cognitive function, is restored by FKBP1b, very possibly via Ca\(^{2+}\) signaling pathways. Accordingly, although tangles are not generally present in animal models of normal brain aging, we suggest that declining FKBP1b function and resulting Ca\(^{2+}\) dysregulation during normal aging in animals recapitulate brain-aging processes in humans that subtly degrade the cytoskeleton and, in susceptible individuals, eventually result in irreversible neurofibrillary tangles and AD.

**References**

Alberini CM, Kandel ER (2014) The regulation of transcription in memory consolidation. Cold Spring Harb Perspect Biol 7:a021741. CrossRef Medline

Backman C, Rose GM, Hooffer BJ, Henry MA, Bartus RT, Friden P, Granholm AC (1996) Systemic administration of a nerve growth factor conjugate reverses age-related cognitive dysfunction and prevents cholinergic neuron atrophy. J Neurosci 16:5437–5442. Medline

Bartus RT, Dean RL 3rd, Fleming DL (1979) Aging in the rhesus monkey: effects on visual discrimination learning and reversal learning. J Gerontol 34:209–219. CrossRef Medline

Benjamin Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Royal Statistical Society, Series B 57:289–300.

Bezprozvanny I, Mattson MP (2008) Neuronal calcium mishandling and the pathogenesis of Alzheimer’s disease. Trends Neurosci 31:454–463. CrossRef Medline

Binder DK, Scharfman HE (2004) Brain-derived neurotrophic factor. Growth Factors 22:123–131. CrossRef Medline

Bizon JL, LaSarge CL, Montgomery KS, McDermott AN, Setlow B, Griffith WH (2009) Spatial reference and working memory across the lifespan of male Fischer 344 rats. Neurobiol Aging 30:646–655. CrossRef Medline

Blalock EM, Chen KC, Sharrow K, Herman JP, Porter NM, Foster TC, Landfield PW (2003) Gene microarrays in hippocampal aging: statistical profiling identifies novel processes correlated with cognitive impairment. J Neurosci 23:3807–3819. Medline

Blalock EM, Geddes JW, Chen KG, Porter NM, Markesbery WR, Landfield PW (2004) Incipient Alzheimer’s disease: microarray correlation analyses reveal major transcriptional and tumor suppressor responses. Proc Natl Acad Sci U S A 101:2173–2178. CrossRef Medline

Blalock EM, Chen KC, Stromberg AJ, Norris CM, Kadish I, Kramer SD, Porter NM, Landfield PW (2005) Harnessing the power of gene microarrays for the study of brain aging and Alzheimer’s disease: statistical reliability and functional correlation. Aging Cell 4:481–512. CrossRef Medline

Blalock EM, Grondin R, Chen KC, Thibault O, Thibault V, Pandya JD, Dowling A, Zhang Z, Sullivan F, Porter NM, Landfield PW (2010) Aging-related gene expression in hippocampus proper compared with dentate gyrus is selectively associated with metabolic syndrome variables in rhesus monkeys. J Neurosci 28: 6058–6071. CrossRef Medline

Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19:185–193. CrossRef Medline

Brib V, Baudry M (2016) Calpains: master regulators of synaptic plasticity. Neuroscientist 23:221–231. CrossRef Medline

Chen KC, Blalock EM, Curran-Rauhut MA, Kadish I, Blalock SJ, Brewer L, Porter NM, Landfield PW (2013) Glucocorticoid-dependent hippocampal transcriptome in male rats: pathway-specific alterations with aging. Endocrinology 154:2807–2820. CrossRef Medline

Curlik DM, Weiss C, Nicholson DA, Disterhoft JF (2014) Age-related impairments on one hippocampal-dependent task predict impairments on a subsequent hippocampal-dependent task. Behav Neurosci 128:676–688. CrossRef Medline

Disterhoft JF, Thompson LT, Moyer JR Jr, Mogul DJ (1996) Calcium-dependent afterhyperpolarization and learning in young and aging hippocampus. Life Sci 59:413–420. CrossRef Medline

Fletcher BR, Hill GS, Long JM, Gallagher M, Shapiro ML, Rapp PR (2014) A fine balance: Regulation of hippocampal Arc/Arg3.1 transcription, trans-
lation and degradation in a rat model of normal cognitive aging. Neurobiol Learn Mem 115:58–67. CrossRef Medline

Forster MJ, Lal H (1992) Within-subject behavioral analysis of recent memory in aging mice. Behav Pharmacol 3:337–349. CrossRef Medline

Furnman JL, Norris CM (2014) Calcineurin and glial signaling: neuroinflammation and beyond. J Neuroinflammation 11:138. CrossRef Medline

Gall CM, Pinkstaff JK, Lauterborn JC, Xie Y, Lynch G (2003) Integrins regulate neuronal neurotrophin gene expression through effects on voltage-sensitive calcium channels. Neuroscience 118:925–940. CrossRef Medline

Gallagher M, Rapp PR (1997) The use of animal models to study the effects of aging on cognition. Annu Rev Psychol 48:339–370. CrossRef Medline

Galvin JE, Ginsberg SD (2005) Expression profiling in the aging brain: a perspective. Aging Res Rev 4:529–547. CrossRef Medline

Gant JC, Chen KC, Norris CM, Kadish I, Thibault O, Blalock EM, Porter NM, Landfield PW (2014) Disrupting function of FK506-binding protein 1b/12.6 induces the Ca(2+)-dependent dysregulation of aging phenotype in hippocampal neurons. J Neurosci 31:1693–1705. CrossRef Medline

Gant JC, Blalock EM, Chen KC, Kadish I, Porter NM, Norris CM, Thibault O, Landfield PW (2014) FK506-binding protein 1b/12.6: a key to aging-related hippocampal Ca2+ dysregulation? Eur J Pharmacol 739:74–82. CrossRef Medline

Gant JC, Chen KC, Kadish I, Blalock EM, Thibault O, Porter NM, Landfield PW (2015) Reversal of aging-related neuronal Ca2+ dysregulation and cognitive impairment by delivery of a transgene encoding FK506-binding protein 12.6/1b to the hippocampus. J Neurosci 35:10878–10887. CrossRef Medline

Gibson GE, Peterson C (1987) Calcium and the aging nervous system. Neurobiol Aging 8:329–343. CrossRef Medline

Gibson GE, Zhang H, Toral-Barza L, Szollosi S, Tofel-Grehl B (1996) Calcium stores in cultured fibroblasts and their changes with Alzheimer’s disease. Biochim Biophys Acta 1316:71–77. CrossRef Medline

Ginsberg SD, Minnick K (2006) Functional genomic methodologies. Prog Brain Res 158:15–40. CrossRef Medline

Graef IA, Mermelstein PG, Stankunas K, Neilson JR, Deisseroth K, Tsien RW, Crabtree GR (1999) L-type calcium channels and GS K-3 regulate the activity of NF-AT in hippocampal neurons. Nature 401:703–708. CrossRef Medline

Greer PL, Greenberg ME (2008) From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. Neurosci 59:846–860. CrossRef Medline

Grundke-Iqbal I, Johnson AB, Wisniewski HM, Terry RD, Iqbal K (1979) The paired helical filaments of Alzheimer’s disease. J Neurochem 69:2087–2103. CrossRef Medline

Huang da W, Sherman BT, Lempicki RA (2009) Systematic and integrative examination of a hypothesis. Aging (Milano) 1:17–34. CrossRef Medline

Kadish I, Van Groen T (2003) Differences in lesion-induced hippocampal plasticity between mice and rats. Neuroscience 116:499–509. CrossRef Medline

Kadish I, Thibault O, Blalock EM, Chen KC, Gant JC, Porter NM, Landfield PW (2008) Hippocampal and cognitive aging across the lifespan: a bienergetic shift precedes cognitive decline and exacerbates parallel memory impairment. J Neurosci 29:1805–1816. CrossRef Medline

Kang CB, Hong Y, Dhe-Paganon S, Yoon HS (2008) FKBP family proteins: immunophilins with versatile biological functions. Neurosignals 16:318–325. CrossRef Medline

Kaptll MG, Feigin A, Bland RJ, Young D, Strybing K, Eidelberg D, During MJ (2007) Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson’s disease: an open label, phase I trial. Lancet 369:2097–2105. CrossRef Medline

Kadowanizha ZS (1989) The role of calcium regulation in brain aging: reexamination of a hypothesis. Aging (Milano) 1:17–34. CrossRef Medline

Kuchibhotla KV, Goldman ST, Lattarulo CR, Wu HY, Hyman BT, Backsa BJ (2008) Abeta plaques lead to aberrant regulation of calcium homeostasis in vivo resulting in structural and functional disruption of neuronal networks. Neuro 59:214–225. CrossRef Medline

Landfield PW (1987) ‘Increased calcium-current’ hypothesis of brain aging. Neurobiol Aging 8:346–347. CrossRef Medline

Landfield PW, Pitler TA (1994) Prolonged Ca2+–dependent hyperpolarizations in hippocampal neurons of aged rats. Science 226:1089–1092. CrossRef Medline

Latimer CS, Brewer LD, Scarcy JL, Chen KC, Popovic J, Krander SD, Thibault O, Blalock EM, Landfield PW, Porter NM (2014) Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats. Proc Natl Acad Sci U S A 111:E4359–4366. CrossRef Medline

Lee YS, Silva AJ (2009) The molecular and cellular biology of enhanced cognition. Nat Rev Neurosci 10:126–140. CrossRef Medline

Lehnart SE, Mongillo M, Bellinger A, Chen BX, Husew S, Reiken S, Wranska A, Drew LJ, Ward CW, Lederer WJ, Kass RS, Morley G, Marks AR (2008) Leaky Ca2+ release channel/ryanodine receptor 2 causes seizures and sudden cardiac death in mice. J Clin Invest 118:2230–2245. CrossRef Medline

Liu X, Betzchauser MJ, Reiken S, Meli AG, Xie W, Chen BX, Arancio O, Marks AR (2012) Role of leaky neuronal ryanodine receptors in stress-induced cognitive dysfunction. Cell 150:1035–1047. CrossRef Medline

Lubeki JJ, Amatrudo JM (2012) Age-related increase of sHAP in prefrontal pyramidal cells of monkeys: relationship to cognition. Neurobiol Aging 33:1085–1095. CrossRef Medline

Lynch G, Baurdy M (1987) Brain spectrin, calpain and long-term changes in synaptic efficacy. Brain Res Bull 18:809–815. CrossRef Medline

Lynch G, Rex CS, Chen LY, Gall CM (2008) The substrates of memory: defects, treatments, and enhancement. Eur J Pharmacol 585:2–13. CrossRef Medline

MacMillan D, McCarron JG (2009) Regulation by FK506 and rapamycin of Ca2+ release from the sarcoplasmic reticulum in vascular smooth muscle: the role of FK506-binding proteins and mTOR. Br J Pharmacol 158:1112–1120. CrossRef Medline

Markowska AL (1999) Sex dimorphisms in the rate of age-related decline in synaptic efficacy and beyond. Brain Res Bull 42:27–34. CrossRef Medline

Matusiewicz D, Griffith WH (2007) Calcium buffering systems and calcium signaling in aged rat basal forebrain neurons. Aging Cell 6:297–305. CrossRef Medline

Murchison D, Griffith WH (2007) Calcium buffering systems and calcium signaling in aged rat basal forebrain neurons. Aging Cell 6:297–305. CrossRef Medline

Murphy GG, Shah V, Helf JW, Silva AJ (2006) Investigation of age-related cognitive decline using mice as a model system: neurophysiological correlates. Am J Geriatr Psychiatry 14:1021–1021. CrossRef Medline
