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FK506-binding protein 1b/12.6: a key to aging-related hippocampal Ca^{2+} dysregulation?

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Abstract

It has been recognized for some time that the Ca^{2+}-dependent slow afterhyperpolarization (sAHP) is larger in hippocampal neurons of aged compared with young animals. In addition, extensive studies since have shown that other Ca^{2+}-mediated electrophysiological responses are increased in hippocampus with aging, including Ca^{2+} transients, L-type voltage-gated Ca^{2+} channel activity, Ca^{2+} spike duration and action potential accommodation. Elevated Ca^{2+}-induced Ca^{2+} release from ryanodine receptors (RyRs) appears to drive amplification of the Ca^{2+} responses. Components of this Ca^{2+} dysregulation phenotype correlate with deficits in cognitive function and plasticity, indicating they may play critical roles in aging-related impairment of brain function. However, the molecular mechanisms underlying aging-related Ca^{2+} dysregulation are not well understood.

FK506-binding proteins 1a and 1b (FKBP1a/1b, also known as FKBP12/12.6) are immunophilin proteins that bind the immunosuppressant drugs FK506 and rapamycin. In muscle cells, FKBP1a/1b also bind RyRs and inhibits Ca^{2+}-induced Ca^{2+} release, but it is not clear whether FKBPs act similarly in brain cells. Recently, we found that selectively disrupting hippocampal FKBP1b function in young rats, either by microinjecting adeno-associated viral vectors containing siRNA, or by treatment with rapamycin, increases the sAHP and recapitulates much of the hippocampal Ca^{2+} dysregulation phenotype. Moreover, in microarray studies, we found FKBP1b gene expression was downregulated in hippocampus of aging rats and early-stage Alzheimer's disease subjects. These results suggest the novel hypothesis that declining FKBP function is a key factor in aging-related Ca^{2+} dysregulation in the brain and point to potential new therapeutic targets for counteracting unhealthy brain aging.

Keywords

Calcium; Ryanodine Receptor; Aging; FKBP1b

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1. Introduction

Recent estimates project that 20% of the population of Western countries will be over 65 years of age by 2030, up from just 10% in the year 2000. Further, such changes in the aging population represent a global trend (Lutz et al., 2008). Because Alzheimer's disease (AD) incidence rises rapidly after age 65, the World Health Organization estimates that 65 million people worldwide will have AD by 2030 and 115 million by 2050. Therefore, it is clear that without therapeutic interventions to slow, reverse or prevent progression of unhealthy brain aging and AD, society will face enormous burdens in caring for the cognitively-dysfunctional elderly. Nevertheless, development of disease modifying interventions will very likely require greater understanding of molecular mechanisms underlying unhealthy brain aging than is yet available. The present article reviews several converging lines of evidence that indicate an important role for neuronal Ca^{2+} dysregulation in unhealthy hippocampal aging, and highlights recent findings that reveal a potential novel molecular mechanism underlying this Ca^{2+} dysregulation.

2. Neuronal calcium (Ca^{2+}) dysregulation with aging

2.1. Ca^{2+} dysregulation in hippocampal neurons and unhealthy brain aging

It has been recognized for some time that the Ca^{2+}-dependent slow afterhyperpolarization (sAHP) that follows a burst of action potentials in hippocampal CA1 pyramidal neurons is larger in aged compared to young-adult animals (Deyo et al., 1989; Disterhoft et al., 1996; Kerr et al., 1989; Landfield and Pitler, 1984; Moyer et al., 1992; Potier et al., 1993). The sAHP is generated by Ca^{2+} influx via voltage-gated Ca^{2+} channels (VGCCs) that activates hyperpolarizing K^+ currents, which in turn dampen postsynaptic excitability. In addition to the larger sAHP, multiple other Ca^{2+}-related electrophysiological processes have since been shown to be increased in hippocampal pyramidal neurons of aged animals, including Ca^{2+} action potential duration, L-type VGCC (L-VGCC) activity, voltage-activated Ca^{2+} transients, long-term depression and action potential accommodation (Brewer et al., 2009; Disterhoft et al., 1996; Foster and Norris, 1997; Moyer et al., 1992; Pitler and Landfield, 1990; Potier et al., 1993; Thibault et al., 2001; Thibault and Landfield, 1996). Importantly, several of these enhanced Ca^{2+}-related functions are correlated with impairment of learning or synaptic plasticity (Disterhoft and Oh, 2007; Disterhoft et al., 1996; Kumar and Foster, 2004; Thibault et al., 2001; Thibault and Landfield, 1996; Tombaugh et al., 2005). Together, these Ca^{2+}-related biomarkers of aging appear to comprise a consistent Ca^{2+} dysregulation phenotype in hippocampal CA1 pyramidal neurons of aging animals. Interestingly, CA1 pyramidal neurons are also among the brain neurons most vulnerable to degeneration induced by AD, ischemia and other pathological conditions (Morrison and Hof, 2002; Wang et al., 2010).

2.2 Ca^{2+}-induced Ca^{2+} release (CICR) from ryanodine receptors (RyRs) on the endoplasmic reticulum (ER)

While Ca^{2+} influx from increased L-VGCC activity apparently is an important source of Ca^{2+} driving hippocampal Ca^{2+} dysregulation, another major source of elevated Ca^{2+} appears to be enhanced Ca^{2+}-induced Ca^{2+} release (CICR) from intracellular Ca^{2+} stores.

CICR is mediated by Ca^{2+} sensing ryanodine receptors (RyRs) on the ER that release additional Ca^{2+} in response to Ca^{2+} influx via plasmalemmal channels. It is well-established that RyRs operate downstream of and in series with L-VGCCs in mediating CICR. L-VGCCs and RyRs are closely juxtaposed in cardiomyocytes (Protasi, 2002; Sedarat et al., 2000) and neurons (Chavis et al., 1996), and interact physically in hippocampus (Berrout and Isokawa, 2009; Kim et al., 2007). To ensure preferential activation by L-VGCCs, RyRs are bound in close apposition to L-VGCCs by junctophilins in a heteromeric junctional membrane complex (Moriguchi et al., 2006; Takeshima et al., 2000). This complex also contains proteins that regulate Ca^{2+} release from RyRs, including FK506-binding proteins 1a and 1b (FKBP1a/1b, also known as FKBP12/12.6), and calmodulin-3 (Brillantes et al., 1994; Jayaraman et al., 1992; Lehnart et al., 2003; Meissner, 2002; Samso et al., 2009; Wagenknecht et al., 1997; Wright et al., 2008; Zalk et al., 2007). CICR functions to rapidly amplify the Ca^{2+} influx that enters via membrane Ca^{2+} channels, and thereby to achieve the high Ca^{2+} concentrations required for activation of some major Ca^{2+}-dependent functions, such as excitation-contraction coupling in muscle or Ca^{2+} signaling in large networks (Roderick et al., 2003; Verkhratsky, 2005). Thus, CICR is well-positioned to modulate Ca^{2+} signaling. On the other hand, if CICR is aberrant, it is also effectively positioned to drive widespread Ca^{2+} dysregulation.

A significant increase in CICR is seen with age in several models of hippocampal Ca^{2+} dysregulation, from longer-term cultured embryonic hippocampal neurons (e.g., Clodfelter et al., 2002) to hippocampal slice neurons of adult rats (Gant et al., 2006; Kumar and Foster, 2005). In a large electrophysiological and Ca^{2+}-imaging study of CA1 neurons across the adult lifespan of male F344 rats, we found that several established biomarkers of Ca^{2+} dysregulation (larger sAHP, greater spike accommodation and increased Ca^{2+} transients) emerge roughly together in the midlife age range (Gant et al., 2006). This is also the age range in which increased L-VGCC activity and impaired cognition first emerge (Aitken and Meaney, 1989; Bach et al., 1999; Fischer et al., 1992; Frick et al., 1995; Kadar et al., 1994; Kadish et al., 2009; Knuttinen et al., 2001; Markowska, 1999; Norris et al., 2010; Thibault and Landfield, 1996). Of particular note in the lifespan study (Gant et al., 2006), is that ryanodine, at concentrations that block RyRs and CICR, essentially erases the aging-related increases on all measured biomarkers of Ca^{2+} dysregulation (Fig. 1), including the increase in Ca^{2+} transients induced by trains of action potentials at 7 Hz (theta). Therefore, much of the aging-related increase in intracellular [Ca^{2+}] during physiologically-relevant stimulation (7 Hz) apparently originates from CICR and likely underlies the age differences in multiple Ca^{2+}-dependent responses of hippocampal CA1 pyramidal neurons. Based on these findings, our initial 'Ca^{2+}-current hypothesis' of Ca^{2+} dysregulation in hippocampal aging (Landfield, 1987) has been extended to incorporate the growing evidence that augmented CICR further amplifies an already increased L-VGCC source of Ca^{2+} and is a major contributing factor to Ca^{2+} dysregulation in brain aging (Gant et al., 2006; Thibault et al., 2007).

2.3 Different patterns of aging-related Ca^{2+} dysregulation in other neuron types and preparations

From the outset of studies on Ca^{2+} dysregulation and brain aging, it has been apparent that manifestations of aging-related Ca^{2+} dyshomeostasis varied widely across neuron types,

regions and preparations (Gibson and Peterson, 1987; Khachaturian, 1984; Michaelis et al., 1984; Peterson and Gibson, 1984), some differing substantially from the hippocampal pyramidal cell Ca^{2+} dysregulation phenotype (Landfield and Pitler, 1984; Thibault et al., 2007). For example, aged rodent dentate gyrus granule neurons are characterized by smaller Ca^{2+} currents, possibly because of elevated intracellular free Ca^{2+} and resultant Ca^{2+}-dependent Ca^{2+} inactivation (Reynolds and Carlen, 89). Rodent cerebellar granule neurons exhibit aging-related increases in resting Ca^{2+}, and lower-amplitude but longer-lasting Ca^{2+} transients from ER release (Kirischuk et al., 1996), perhaps linked to altered mitochondrial function (Toescu and Verkhratsky, 2004). Furthermore, an aging-related increase in Ca^{2+} buffering capacity has been found consistently in basal forebrain neurons (see Murchison and Griffith, 2007; Murchison et al., 2009), and may also occur in hippocampal CA1 neurons (Oh et al., 2013). Conversely, aging-related reductions in apparent Ca^{2+} buffering capacity (e.g., calbindin, mitochondria) have been found, in the rat perirhinal cortex (Moyer et al., 2011) and some other regions (Brown et al., 2004). Recent work on mouse hippocampal CA3 interneurons (Lu et al., 2011) found no aging effects on sAHPs, intracellular Ca^{2+} transients and resting free Ca^{2+} under baseline conditions. However, during kainate activation these processes exhibited aging effects that resembled those noted above in hippocampal pyramidal neurons. Clearly, resolving whether this variation in Ca^{2+}-related manifestations of brain aging reflects modifications induced by different neuronal architectures, preparations or physiological processes acting on a common underlying mechanism of Ca^{2+} dysregulation, or instead indicates that there are multiple cell-type-specific mechanisms of neuronal Ca^{2+} dyshomeostasis, will require considerable additional study.

2.4 Altered Ca^{2+} regulation in mouse models of Alzheimer's disease

As noted, aging is the major risk factor for idiopathic AD and it therefore seems reasonable that mechanisms of unhealthy brain aging related to conversion of normal aging to AD might well continue to act in AD brain. Accordingly, it is interesting that elevated intracellular Ca^{2+} release in neurons also has been found in transgenic mouse models of AD (Chakroborty et al., 2009; Leissring et al., 2000; Mattson et al., 1998; Stutzmann et al., 2006; Supnet and Bezprozvanny, 2011; Tu et al., 2006). Such results are generally similar to those described above for studies of normal aging in hippocampal neurons, in that Ca^{2+} dyshomeostasis was in part manifested by altered CICR. On the other hand, in contrast to normal aging, hippocampal L-VGCC activity was recently found to be reduced in a transgenic AD model compared to wild-type controls, possibly because of Ca^{2+} inactivation (Thibault et al., 2012).

Substantial evidence from human AD brain samples, notably an increase in calpain activity (Nixon, 2003), also suggests a role for Ca^{2+} dyshomeostasis in AD or other neurodegenerative conditions (Corona et al., 2011; Fedrizzi and Carafoli, 2011; Goussakov et al., 2010). Furthermore, studies of brain samples from AD and MCI patients (Bruno et al., 2012) found that mRNA for RyR2 was elevated in temporal cortex in the MCI group compared to cognitively-intact controls. Potentially decreased Ca^{2+} buffering, indicated by reduced calbindin immunoreactivity, has been found in basal forebrain regions of human AD samples (Riascos et al., 2011). In addition, although the results are controversial, several

clinical trials have suggested that treatment with Ca^{2+} channel blockers may delay the onset of dementia (Forette et al., 2002; Goodison et al., 2012; Khachaturian et al., 2006; Watfa et al., 2010).

3. Molecular mechanisms

As discussed, multiple Ca^{2+}-related processes have been implicated in brain aging, among which L-VGCCs and RyRs have been identified as likely primary sources of excess Ca^{2+} in aged animal hippocampal CA1 neurons. Despite these substantial advances in elucidating aging effects on neuronal physiology, little is yet known about the molecular bases of these physiological alterations or specifically, about the molecular mechanisms underlying age-related alterations in L-VGCC or RyR activity. There is some evidence of modest upregulation of the less common isoform (CaV1.3) of the L-VGCC pore-forming subunit (Chen et al., 2000; Herman et al., 1998; Veng et al., 2003), and conditional knock down of CaV1.3 was found to reduce the sAHP of mice (Gamelli et al., 2011). Nevertheless, our gene array analyses (see below) have not confirmed upregulation of expression, at the mRNA level, of any L-VGCC pore-forming subunits (Rowe et al., 2007), and to date have found only modest late-life increases in RyR expression (Kadish et al., 2009). Therefore, at this point changes in brain L-VGCC or RyR expression do not appear to be compelling explanations for their increased activity with aging.

3.1 Whole-genome expression profiles associated with hippocampal aging and cognitive decline

In parallel with the electrophysiological/imaging studies, we have conducted a series of microarray analyses aimed at identifying whole-genome expression profiles associated with aging-related cognitive impairment, including three studies of F344 rat hippocampal expression (Blalock et al., 2003; Kadish et al., 2009; Rowe et al., 2007). These studies differed from one another somewhat in the age of animals used and in time points of gene expression analysis following Morris water maze (MWM) training. However, the Kadish *et al.* (2009) study examined hippocampal expression changes and cognition across the adult lifespan and therefore closely parallels our age course study of hippocampal electrophysiological and $[Ca^{2+}]_i$ changes (Gant et al., 2006). In the lifespan gene array study (Kadish et al., 2009), we examined the age course of changes in hippocampal aging-related genes, and pathways they represented, correlating these with cognitive function at 5 age points of the rat adult lifespan (3-, 6-, 9-, 12- and 23-months-old). Statistically well-powered groups, with one chip per animal were employed, allowing detection of modest expression differences with high reliability. False discovery rates were held down by use of pre-analytic filtering algorithms, well-powered tests and pathway analyses, allowing the studies to take advantage of the discovery power of microarrays while mitigating both Type I and Type II error (Blalock et al., 2003; Blalock et al., 2005; Peng et al., 2003).

The lifespan analysis identified multiple brain processes that begin to change early in aging, and consequently might be strong candidates for initiators of unhealthy brain aging cascades that induce onset of cognitive decline. Of particular interest in the present context, however, data mining subsequent to publication revealed aging-related changes in a group of genes

encoding immunophilins and other proteins relevant to regulation of CICR, as described below.

3.2 The FKBP-Ca^{2+} regulating pathway: a possible molecular mechanism underlying Ca^{2+} dyshomeostasis

FK506-binding proteins (FKBP) 1a and FKBP1b (also known as FKBP12 and 12.6, respectively) are low molecular weight members of the FKBP family of immunophilins, proteins that bind the immunosuppressant drugs FK506 and rapamycin. Many immunophilins exhibit peptidyl-prolyl isomerase activity and function as protein chaperones and stabilizers (Eitoku et al., 2008; Jakob et al., 2009; Kang et al., 2008; Marks, 1997). In myocytes, FKBP1a and FKBP1b also play a major role in Ca^{2+} regulation, binding and stabilizing RyRs in the closed state and inhibiting CICR from sarcoplasmic reticulum stores. In cardiomyocytes, genetic depletion of FKBP1b results in Ca^{2+} leakage from RyRs and cardiac failure (Lehnart et al., 2003; Zalk et al., 2007). In addition, treatment with rapamycin displaces FKBPs from RyRs and increases Ca^{2+} release from intracellular stores (Lehnart et al., 2003; Long et al., 2007).

However, there have been few studies on the roles of FKBPs in brain neurons. We were prompted to investigate this pathway in neurons by our findings implicating RYRs in brain Ca^{2+} dysregulation (Gant et al., 2006 and Fig. 1) and by our unexpected observation that rapamycin increased Ca^{2+} currents (unlike FK506, which inhibits Ca^{2+} currents) (Norris et al., 2002; Norris et al., 2010). Moreover, our microarray analyses showed that hippocampal *Fkbp1b* gene expression is downregulated with aging; *Fkbp1b* expression begins to decline early in the lifespan and continues to drop through mid- and late-life, when cognitive deficits typically emerge. *Fkbp1a* also declines with aging although its decrease is somewhat more variable (Fig. 2). Together, these lines of evidence raised the possibility that FKBP pathways might also regulate CICR in brain neurons and that their decline might play a critical role in Ca^{2+} dysregulation in aging.

3.3 Testing predictions of a novel hypothesis implicating FKBP-Ca^{2+} release pathways in aging-related brain Ca^{2+} dysregulation

Accordingly, we undertook studies to test specific predictions of the hypothesis that the FKBP-RyR pathway is a major player in aging-related hippocampal Ca^{2+} dysregulation. To test the possibility that a decline of FKBP function in hippocampal neurons might play a role in aging-related Ca^{2+} dysregulation, we used two distinct but complementary approaches to disrupt FKBP1b function. In one, hippocampal embryonic cell cultures or hippocampal slices from young-adult rats were exposed to rapamycin, which displaces FKBPs from RyRs. In the second approach, siRNA *(siFkbp1b)* was used to selectively knock down *Fkbp1b* expression *in vitro*; for *in vivo* studies, adeno-associated viral (AAV) vectors bearing *shRNA* constructs were microinjected into the hippocampus to knock down *Fkbp1b* expression (Gant et al., 2011).

3.4 In vitro rapamycin treatment and siRNA knockdown of FKBP1b

In the *in vitro* studies, incubation of hippocampal cell cultures with rapamycin for 1 hour had no effect on VGCC currents, but larger currents were observed following 24 or 96 hours

of incubation (Fig. 3). The L-VGCC blocker, nimodipine reversed the effect of rapamycin on VGCC current, indicating that the rapamycin-induced increase in current was primarily mediated via L-type VGCCs. Moreover, following 3–4 days of *in vitro* knockdown of FKBP1b by targeted siRNA, VGCC activity was enhanced, much as it was by rapamycin (Fig. 3). Treatments with scrambled siRNA or siRNA vehicle had no effect on Ca^{2+} currents. Confirming efficacy of knockdown, qPCR analyses showed that FKBP1b mRNA was significantly reduced following incubation with *siFkbp1b* (Gant et al., 2011).

3.5 In situ rapamycin treatment of hippocampal slices and in vivo shRNA knockdown of FKBP1b

To determine the effect of rapamycin on Ca^{2+} responses in adult neurons, we incubated young-adult rat hippocampal slices in rapamycin for electrophysiological recording and Ca2+ imaging. These studies revealed that rapamycin substantially enhanced both the sAHP and neuronal Ca^{2+} transients, especially the CICR component of the Ca^{2+} transient. Perfusion of the recording chamber with high concentrations of ryanodine, sufficient to block ryanodine-dependent CICR, reversed the effects of rapamycin on the sAHP and Ca^{2+} transients.

In order to selectively manipulate gene expression in defined hippocampal regions of adult rats, we conducted a series of comparative studies to determine the ability of various microinjected adeno-associated virus (AAV) serotypes expressing green fluorescent protein (GFP) to transduce pyramidal neurons in the hippocampus. GFP expression showed that all serotypes successfully transduced hippocampal pyramidal neurons and that expression of GFP was limited to the hippocampus proper. Serotype 8 exhibited the least amount of longitudinal spread, while serotypes 1 and 9 exhibited longitudinal spread sufficient to transduce neurons in the hippocampus from the extreme rostral to caudal portions. Serotype 1 was chosen as the preferred serotype for further studies. Our target injection site was *Stratum Oriens* at the peak of the CA1 pyramidal cell layer. Figure 4 shows sections confirming viral delivery of the GFP transgene to hippocampal CA1 neurons and illustrates that the vector did not substantially cross the hippocampal fissure or the midline following unilateral injection of AAV.

We then conducted a major study testing predictions of the FKBP-Ca^{2+} dysregulation hypothesis, employing unilateral hippocampal knockdown of FKBP1b *in vivo.* In this study, one hippocampus *(ipsilateral)* of young-adult rats was microinjected with AAV expressing *shFkbp1b,* while the other hippocampus *(contralateral)* received either empty-vector control AAV(AAV-0) or was not treated (these two control conditions did not differ on any variable and were combined statistically). Approximately, four-to-five weeks after *shFkbp1b* injection, intracellular electrophysiological recordings of the sAHP were obtained from CA1 pyramidal neurons in acute hippocampal slices from both transduced *(ipsilateral)* and control *(contralateral)* sides of 9 animals.

As shown in Figure 5, the Results of the electrophysiological studies were dramatic. sAHP measures were obtained from a total of 17 neurons in slices from the ipsilateral and 17 from the contralateral hippocampi of the 9 injected young rats. Statistical analysis revealed that sAHP amplitude and duration were increased by approximately 100% in the transduced

hippocampus. Moreover, the sAHPs of control neurons were similar to those typically observed in young rats, whereas values from knockdown side neurons were similar to those observed in aged rats in several prior studies (e.g., Fig. 1). Our qPCR and immunohistochemical data from this knockdown study clearly showed that FKBP1b knockdown was achieved in hippocampus at both the gene (25–35% by qPCR) and protein (Fig. 6) levels.

In sum, rapamycin or FKBP1b knockdown in young rat neurons closely recapitulated patterns of Ca^{2+} dysregulation seen with aging, findings that are clearly consistent with a key prediction of the hypothesis that FKBP1b disruption is an important molecular mechanism underlying aging-related Ca^{2+}-dysregulation in the hippocampus (Gant et al., 2011). It should also be noted that extensive studies are presently underway testing the converse prediction, namely, that in vivo overexpression of *Fkbp1b* in hippocampus of aging rats can reverse the aging-related pattern of Ca^{2+} dysregulation. Initial results are highly consistent with this prediction.

4. Interactions of FKBP1b/1a with multiple targets

4.1 Do FKBPs regulate L-VGCCs as well as RyRs in the brain?

As described above, it has been well established that FKBPs inhibit Ca^{2+} release from RyRs in muscle cells (Zalk et al., 2007). However, the studies of the effects of disrupting FKBP1b on the ryanodine-sensitive sAHP and Ca^{2+} transient in hippocampus (Fig. 5) provide essentially the first evidence that FKBPs inhibit RyR-mediated CICR in brain neurons as well as in myocytes. Moreover, the findings that FKBP1b apparently inhibits VGCC current (Fig. 3) provides the first evidence in any cell type that FKBP1b inhibits Ca^{2+} entry via VGCCs as well as intracellular release from RyRs (Gant et al., 2011). It is not clear what pathway might mediate FKBP regulation of VGCCs. The L-VGCC lies upstream of the RyR that is directly bound by FKBPs in the VGCC-RyR circuit that amplifies Ca^{2+} through CICR (see Berridge, 1997; Verkhratsky, 2004). Therefore, it is feasible that FKBPs regulate Ca^{2+} currents through their actions on RyRs and unrecognized secondary retrograde effects from RyRs to VGCCs. Nevertheless, a more parsimonious explanation seems to be that FKBPs regulate VGCC activity directly, through an as yet undetermined interaction.

4.2 Inhibition of the mTOR pathway by FKBPs

FKBP1a and FKBP1b interact with cellular pathways in addition to the RyR complex. In particular, FKBPs also tonically inhibit the mTOR pathway that plays an important role in cell growth and plasticity in the brain (Hoeffer et al., 2008; Jacinto and Hall, 2003). Thus, a decline in FKBP1b function with aging, as in our proposed model (Fig. 7) should result in reduced inhibition and increased activity of both the RyR and mTOR pathways. Rapamycin, on the other hand, has opposite effects on the FKBP-RyR and FKBP-mTOR interactions, disrupting FKBP inhibition of RyRs, but strengthening FKBP-mediated inhibition of mTOR. Conceivably, this rapamycin-enhanced mTOR inhibition may account for why rapamycin has been reported to exert some anti-aging-like effects, including extension of lifespan (Caccamo et al.; Harrison et al., 2009; Wilkinson et al., 2012). That is, although BDNF, insulin and other growth factors that mediate neurite extension, plasticity and

neurogenesis signal through mTOR (Lynch et al., 2008; Scharfman and MacLusky, 2006), beneficial effects of inhibiting mTOR may result because of excessive mTOR activation with aging. Nevertheless, rapamycin's anti-aging-like strengthening of FKBP-dependent inhibitory actions on mTOR appear unrelated to rapamycin's aging-like weakening of FKBP-dependent inhibition of RyR-mediated Ca^{2+} release, and likely do not obviate the interpretation that rapamycin disrupts the FKBP-RyR interaction (see model-Fig. 7).

5. A molecular model for aging-related Ca^{2+} dysregulation in hippocampus

Together, the data provide increasingly compelling support for the hypothesis that an aging-related decline in function/expression of hippocampal FKBP1b (and possibly, FKBP1a) is a key factor in development of the hippocampal Ca^{2+} dysregulation phenotype (Gant et al., 2011). This decline may result from decreased expression and/or posttranslational modification. Figure 7 schematically illustrates the model, incorporating FKBP1b regulation of VGCCs as well as RyRs, and shows putative aging effects on multiple target pathways of FKBPs. Also illustrated is the opposite regulation of the FKBP-RyR and FKBP-mTOR pathways by rapamycin.

5.1 Possible causes of age-related decline in FKBP1b function

The finding that disrupting hippocampal FKBP function can induce the Ca^{2+} dysregulation phenotype is consistent with a model in which similar disruption occurs with aging and raises the question of what factors might trigger an initial aging-related decline in FKBP function. One possibility is that a decrease in the biosynthesis of energy-expensive proteins, notably including FKBPs, might result from a metabolic shift that appears to develop in neurons and glia of the hippocampus during aging of females (Brinton, 2008; Yau and Seckl, 2012) and males (Kadish et al., 2009; Rowe et al., 2007). These metabolic alterations in turn may depend on age-related variations in endocrine status, as some hormones (e.g., estrogens and progestins) appear to interact strongly with brain aging and brain metabolism (Brewer et al., 2009; Foster, 2012; Simpkins and Singh, 2008; Sohrabji and Bake, 2006; Xin et al., 2002). Additionally, adrenal stress hormones (glucocorticoids) exert major effects on metabolism and have long been linked to brain aging (Porter and Landfield, 1998). The impact of glucocorticoids on the brain is altered with aging (Blalock et al., 2010; Chen et al., 2013; Holmes and Seckl, 2006; Landfield et al., 2007). Glucocorticoids also enhance the Ca^{2+}-dependent sAHP in the hippocampus (Joels and de Kloet, 1989; Kerr et al., 1989), consistent with an effect on FKBPs. Similarly, mineralocorticoids have been shown to decrease cardiac FKBP1b expression and elevate RyR-mediated Ca^{2+} release (Gomez et al., 2009). Thus, it appears plausible that hormonal and/or metabolic changes may contribute to aging-related declines in FKBP expression/function. Regardless of the causal factors, however, declining FKBP function during brain aging appears to provide important new clues in the search for novel targets that can alter the progression of unhealthy brain aging and/or Alzheimer's disease.

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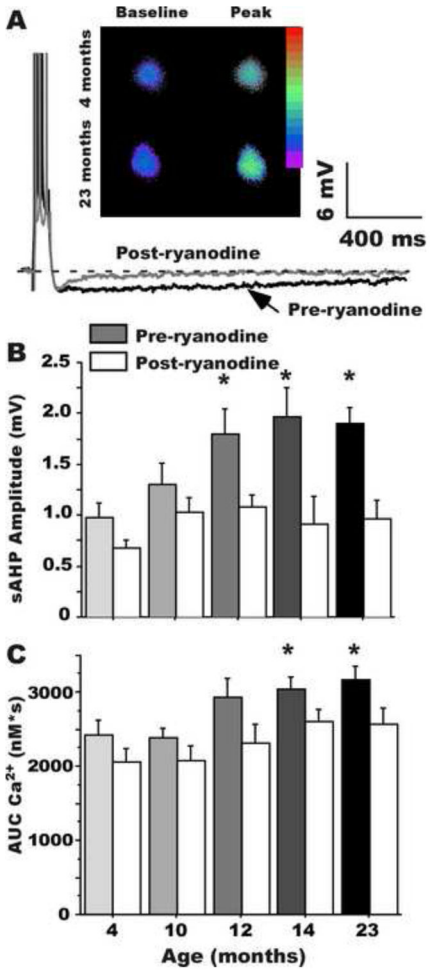

Figure 1. The AHP and intracellular Ca^{2+} are altered by aging

A: Example of the sAHP in hippocampal neurons from a young and an aging rat, showing the increased AHP magnitude with aging (darker trace); also shown are examples of the increase in intracellular Ca^{2+} transients (**inset**). Age-related increases in sAHP amplitude (**B**) and Ca^{2+} transients (**C**) begin by 12 months-of-age and are blocked by ryanodine, indicating the increases depend on CICR from RyRs (* $p < .05$). (From Gant et al., 2006)

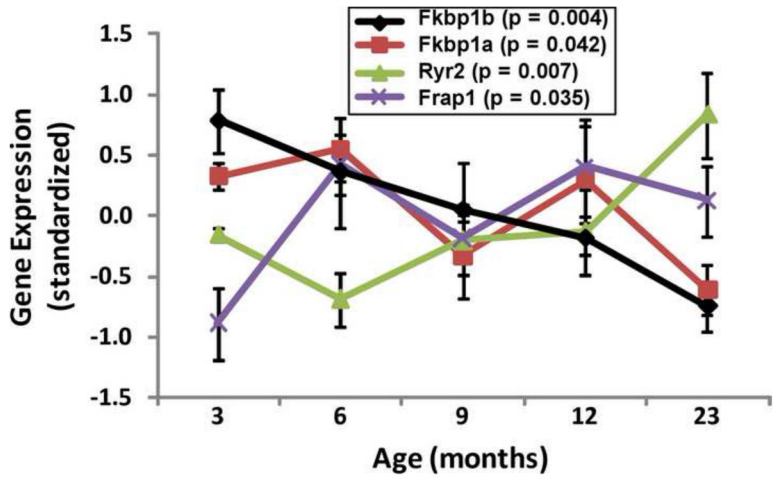

Figure 2. Age-dependent changes in expression of Fkbp1b, Fkbp1a, Ryr2, and Frap1/mTOR mRNA

(From data in Kadish et al., 2009)

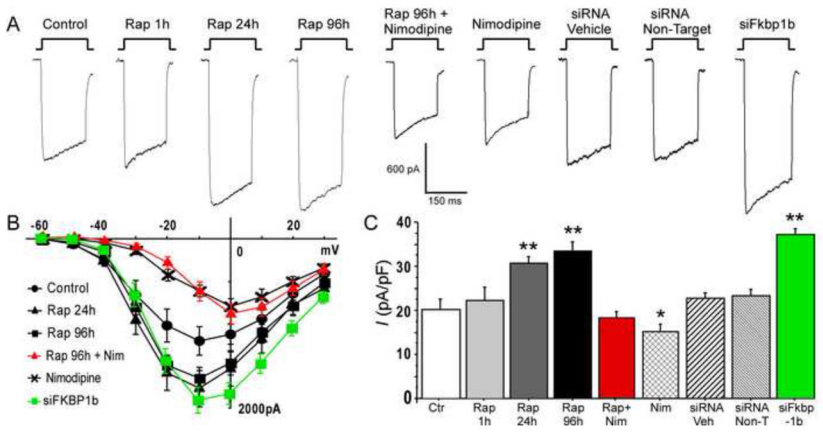

Figure 3. Knockdown of FKBP1b or treatment with rapamycin (Rap) increased Ca^{2+} channel current in cultured hippocampal neurons

A, Representative whole-cell patch-clamp current traces from each experimental condition; B, Mean current/voltage (I/V) relationships for 6 of the 9 experimental conditions. The I–V curves for Rap 1h, siRNA Veh and siRNA Non-Target were not different from the control condition and are omitted for illustrative clarity. Exposure to *siFkbp1b* for 96 h or to rapamycin for 24/96 h induced enhancement of VGCC current. Treatment with siFkbp1b or with rapamycin altered the amplitude but not the voltage dependence of Ca^{2+} current, whereas nimodipine (Nim) shifted peak current to more positive voltage; C, Means +/− S.E.M. of peak Ca^{2+} current density for the 9 conditions. Asterisks indicate significant differences from the control condition (* $p < 0.05$ and **$p < 0.0001$). n = control (18), Rap 1h (4), Rap 24h (20), Rap 96h (20), Rap + Nim (10), Nim (12), siRNA Veh (23), siRNA Non-Target (19), *siFkbp1b* (23). (From Gant et al., 2011)

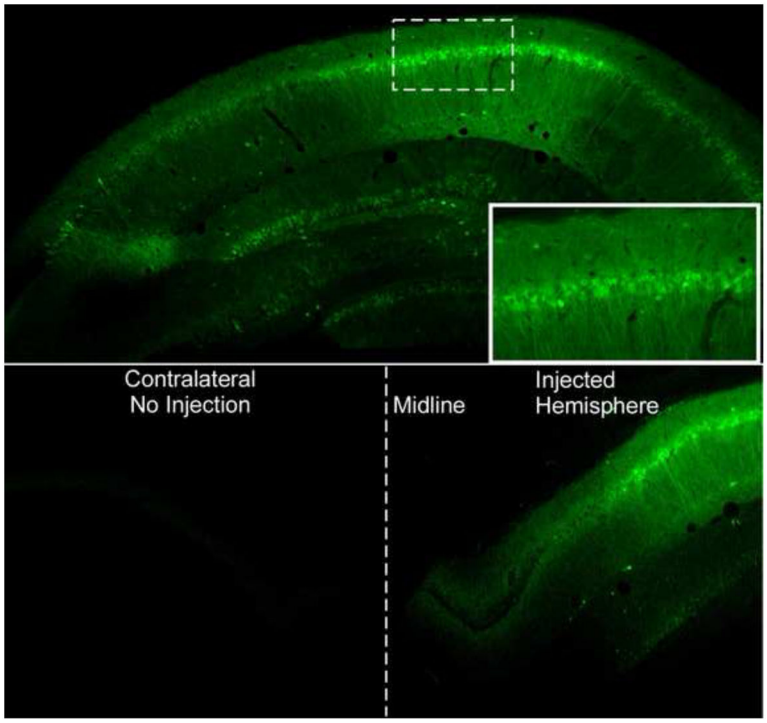

Figure 4. Green Flourescent Protein (GFP) expression in the hippocampus

A: GFP expression in the hippocampus following infusion with serotype 2.1 AAV expressing GFP. Note the high level of expression in the pyramidal cells corresponding to the CA regions. Solid line box inset is shows close-up of pyramidal cells expressing GFP from the CA1 region (Dotted box). **B:** Note that GFP expression is only found in the hippocampus that was injected during a unilateral injection. The contralateral side within the same animal contained no cells expressing GFP. Midline of the hemispheres is denoted with a dotted line.

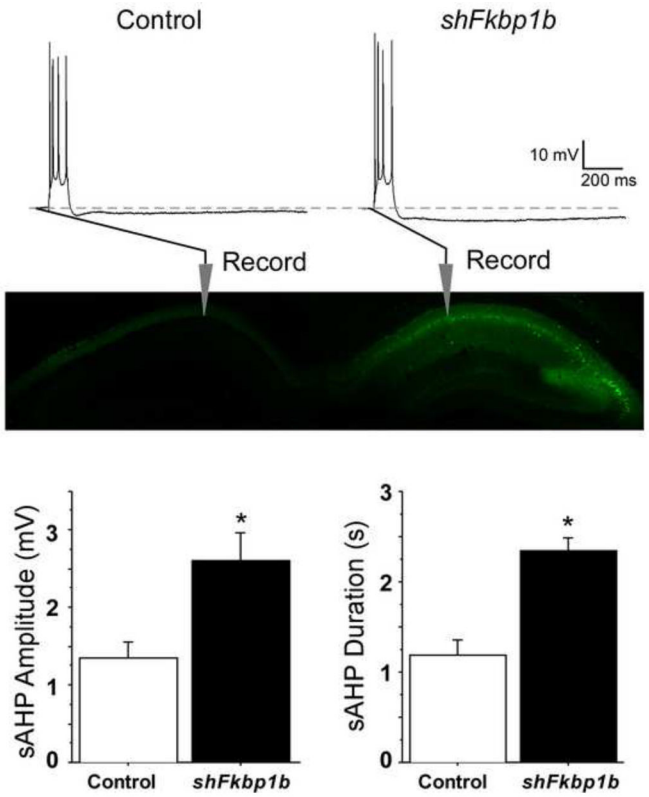

Figure 5. Knockdown of FKBP1b *in vivo* enhanced the slow AHP

Top, Representative sharp electrode intracellular recordings showing four triggered action potentials, followed by an AHP (dashed line indicates baseline) in CA1 neurons from slices of a control (left) and an AAV–*shFkbp1b*-injected (right) hippocampus. Middle, Fluorescent imaging in an animal receiving a similar unilateral AAV injection expressing GFP only, showing strong expression in the injected, but not the contralateral hippocampus, and also illustrating general placement of recording pipettes in the pyramidal neuron somal layer (stratum pyramidale) of field CA1. Bottom, sAHP amplitude (left) and duration (right) were significantly increased by FKBP1b knockdown (*shFkbp1b* injection) (*$p < 0.0025$ for either variable, *t* test; $n = 17$ neurons per group). (From Gant et al., 2011)

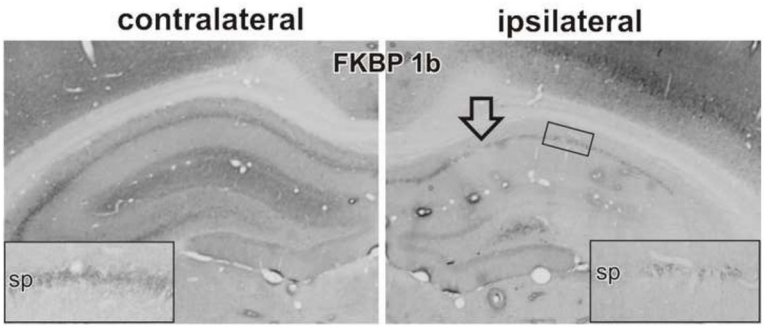

Figure 6. *shFkbp1b* mediated knockdown of *Fkbp1b* validated at the protein level

In vivo direct hippocampal *shFkbp1b* injection into one hemisphere (ipsilateral) is compared to the non-injected contralateral hemisphere. To validate knockdown at the protein level, immunohistochemical staining of FKBP11b/12.6 was performed. Arrow indicates injection site. Inset shows magnified view of CA1 region. (Modified from Gant et al., 2011)

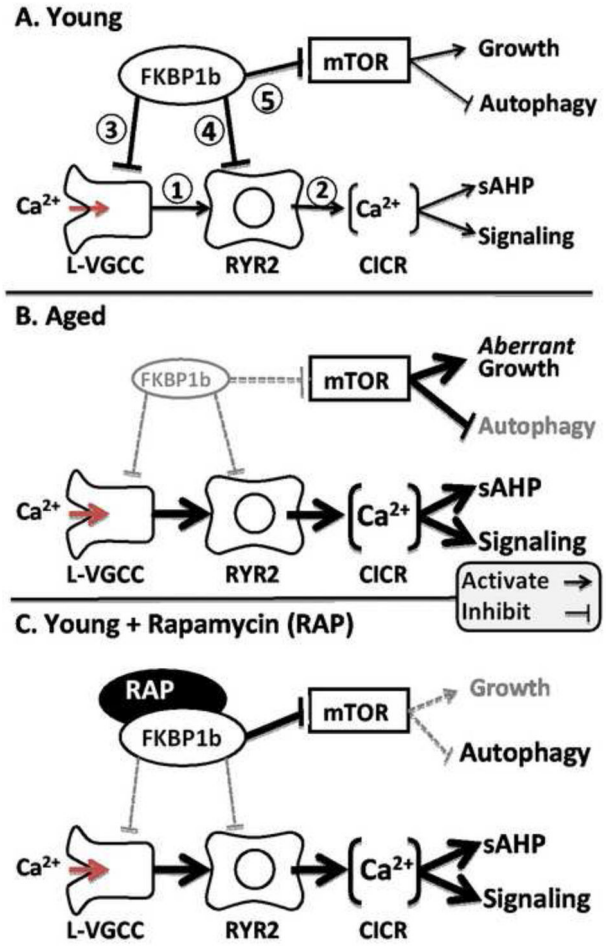

Figure 7. Working model of FKBP1b's role in aging-related Ca^{2+} dyshomeostasis

A: In young subjects, neuronal FKBP1b exerts strong tonic inhibitory effects on two pathways, the Ca^{2+} regulatory pathway and the mTOR signaling pathway. In the Ca^{2+} regulatory pathway, FKBP1b inhibits cytosolic Ca^{2+} rises generated by extracellular influx via L-VGCCs (1) and intracellular release from RYRs (2), by inhibiting both channel types directly (3 & 4). In the mTOR pathway, FKBP inhibition of mTOR (5) helps maintain the balance between growth-stimulating and autophagy-suppressing effects of mTOR. **B:** With aging (mimicked by FKBP1b knockdown), a decline of FKBP1b expression/function leads to weakened inhibition of both pathways, and concomitant increases in both Ca^{2+} and mTOR signaling. Increased Ca^{2+} signaling results in dampened neuronal excitability and function, whereas mTOR disinhibition leads to aberrant increases in growth signaling as well as decreased autophagy. **C:** Rapamycin in young subjects exerts opposite effects on FKBP inhibition in the two pathways, mimicking aging's effect of weakened FKBP inhibition in the Ca^{2+} pathway **(B),** but paradoxically augmenting FKBP inhibition of the mTOR pathway (C). (From Gant et al. 2011)