Published in final edited form as:

Behav Brain Res. 2017 March 30; 322(Pt B): 206–211. doi:10.1016/j.bbr.2016.07.042.

CREB, cellular excitability, and cognition: implications for aging

Xiao-Wen Yu^a, M. Matthew Oh^a, and John F. Disterhoft^{a,b}

^aDepartment of Physiology, Feinberg School of Medicine, Northwestern University, 303 E Chicago Avenue, Chicago, IL, 60611, U.S.A

Abstract

Humans and laboratory animals display cognitive deficits as they age. However, there are currently no effective therapies available to treat these deficits, as the underlying mechanisms are poorly understood. Studies using pharmacological compounds have found a link between cognitive performance and the intrinsic cellular excitability of CA1 hippocampal neurons. Therefore, it is of great interest to identify molecular regulators that may be influencing both cognition and neuronal excitability, which could be changed with age. One possible regulator is the transcription factor cAMP response element binding-protein (CREB). In young adult animals, manipulation of CREB activity has resulted in modulation of both cognitive performance on behavioral tasks, and neuronal excitability. While evidence is sparse, studies also point to a dysfunction in CREB signaling with aging. We propose that CREB may be a viable therapeutic target for the treatment of age-related cognitive deficits, along with potential experiments to test this hypothesis.

Keywords

Hippocampus; Aging; Cognition; CA1; CREB

Cognitive and biophysical deficits in aging

Like humans, laboratory animals display impairments in their ability to learn and remember as they age. However, there are currently no treatments for these cognitive deficits, as their underlying cause is not well understood. In laboratory rats, age-related cognitive deficits have been seen in tasks that utilize spatial memory, such as the Barnes circular maze and the Morris water maze, as well as tasks that require complex temporal processing, such as trace associative conditioning [1]–[4]. Along with cognitive impairments, numerous studies have found that aged animals have neurons that are intrinsically less excitable than those from young animals. Specifically, pyramidal cells from area CA1 of dorsal hippocampus exhibit these changes [5]–[9]. One measure of neuronal excitability is the post-burst afterhyperpolarization (AHP). This is a hyperpolarization that occurs after the neuron has

^bCorresponding author: John Disterhoft, Department of Physiology, Feinberg School of Medicine, Northwestern University, 303 E Chicago Avenue, Chicago, IL, 60611, U.S.A. jdisterhoft@northwestern.edu, Phone: 312-503-7982.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

fired a burst of action potentials. By bringing the neuron further from action potential threshold, a larger AHP makes a neuron less likely to fire again. After an animal successfully learns a task, the AHPs of their CA1 pyramidal neurons are reduced and the neurons are more excitable [5]. Thus, the reduced intrinsic excitability seen in aged animals is thought to be linked to their cognitive impairments.

Fortunately, several compounds have been found which can increase intrinsic excitability of CA1 pyramidal neurons. These compounds are very promising as when given *in vivo*, they also improve performance on behavioral tasks. One example is the L-type calcium channel blocker nimodipine. When applied onto neurons *in vitro*, both young and, especially, aged CA1 pyramidal cells show reduced AHP and spike-frequency accommodation [10]. Furthermore, when nimodipine is given systemically, it facilitates aged animals' ability to learn trace eyeblink conditioning [11]. Galantamine (Razadyne®), a cholinesterase inhibitor, is another example: it increases excitability of CA1 pyramidal neurons *in vitro*, and improves acquisition of trace eyeblink conditioning when delivered *in vivo* to aged animals [12]. In clinical trials, these treatments have proven to be promising options for treatment of various age-related diseases including vascular dementia, chronic cerebrovascular disorder (nimodipine), and Alzheimer's Disease (Razadyne®) [13]. Nilvadipine, an L-type calcium channel blocker related to nimodipine, is also the focus of a current European clinical trial for treatment of Alzheimer's disease. These findings suggest a tight link between the intrinsic excitability of CA1 pyramidal neurons, and an animal's ability to learn and remember. Therefore, it is important to identify the underlying molecular pathways that are disrupted with age, and underlie these changes on both the biophysical and cognitive level.

CREB modulates cognition and excitability in young adult animals

The transcription factor cAMP response element binding-protein (CREB) has been shown to modulate both cognition, and cellular excitability in young adult animals. Long known for its role in memory formation [14]–[17], CREB is activated by phosphorylation at serine133 (pCREB), after which it can dimerize and stimulate transcription of downstream target genes such as BDNF and c-fos [14], [16], [18], [19].

Numerous studies manipulating activity of CREB have demonstrated its crucial role in memory formation [14]–[17], [20], [21], and many comprehensive reviews have been written about it e.g. [22]–[25]. Therefore, this review will highlight a few of the studies. Studies preventing activity of CREB have shown just how important it is for memory formation. Dash et al. were the first to show that blocking CREB function impaired memory [15]. When cAMP-responsive element (CRE) was injected into the nucleus of Aplysia sensory neurons, long-term facilitation normally induced by serotonin was blocked, while short-term facilitation was intact. This showed that by injecting exogenous CRE, CREB's normal binding site, CREB could no longer bind to CRE sites on DNA and did not give the downstream transcription needed for expression of long-term facilitation. Guzowski and McGaugh demonstrated a similar finding by using an antisense oligodeoxynucleotide against CREB in rats [20]. CREB antisense oligodeoxynucleotides were injected into dorsal hippocampus and caused a 36% reduction in CREB protein levels. When rats were injected prior to training on the Morris water maze, acquisition of the task was not impaired, nor was

short-term memory in a retention test carried out 2 hours later. However, a long-term memory test given 48 hours later showed an impairment only in the antisense-injected animals. This indicated that reducing CREB levels impaired long-term memory on a spatial task. A CREB knock-out mouse line was also generated [21]. When these mutants were trained in fear conditioning, they showed very little freezing to context or tone, both in tests of short- and long-term memory. When these mutants were trained on Morris water maze, they had slight impairments in acquisition, and showed no preference for the target quadrant during the probe trial. When long-term potentiation (LTP) experiments were conducted on slices made from these mice, it was found to be small in amplitude, and short-lasting. These results suggest that CREB knock-out results in impairments in memory as well as synaptic plasticity/neuronal excitability. Transgenic mice expressing KCREB, a dominant negative CREB that reduces affinity of normal CREB for DNA, were impaired on both acquisition and retention of the Morris water maze task, and for object recognition [26]. Another transgenic line expressed a mutant CREB where serine 133 was replaced with an alanine to prevent activation of CREB [27]. These mice were impaired in long-term memory in fear conditioning, but short-term memory was left intact. These studies show that reducing CREB activity in different ways result in impairments in memory, typically long-term.

Increasing CREB activity using exogenous compounds, or via transgenic and viral means has been found to facilitate performance on behavioral tasks testing for cognition. When rats received systemic rolipram, a phosphodiesterase inhibitor, for 5 days, hippocampal levels of CREB and CREB phosphorylated at S133 (pCREB) were increased, and memory for fear conditioning was enhanced [28]. Suzuki et al. [29] generated transgenic mice whose CREB had increased affinity for either PKA, which had increased likelihood of being phosphorylated (CREB^{Y134F}), or for CREB-binding protein (CREB-DIEMDL), CREB's co-activator. These mice showed facilitation in long-term memory for social recognition, fear conditioning, Morris water maze, and passive avoidance, all the while showing no differences in short-term memory or learning. Another transgenic mouse line expressed constitutively active VP-16 CREB (caCREB) in the amygdala [30]. This resulted in strong memory for contextual and cued fear conditioning. These studies in rats and transgenic mice indicate that activating CREB results in increased transcription of downstream genes, and facilitates cognition in the form of memory.

While transgenic animals are useful tools, since their gene expression is altered from birth, it can be difficult to tease apart effects of the genetic alteration alone, from their potential effects on developmental processes. Thus, viral vector delivery of genes has the advantage of using animals that develop normally. One example used the Sindbis virus to express CREB^{Y134F} in CA1 hippocampal cells of mice. This resulted in increased contextual fear memory, mirroring previous results found in transgenic mice with the same mutation [31]. In an elegant experiment using HSV to overexpress wild-type CREB, Sekeres et al. [32] demonstrated the necessity and sufficiency of CREB for long-term memory. HSV-CREB was injected into either wild-type mice or CREB knock-out mice, that were then given weak Morris water maze training. In both cases, long-term spatial memory was observed. Josselyn et al. [33] also observed long-term memory with a weak training protocol for fear conditioning (which normally only produces short-term memory) when HSV-CREB was injected into the amygdala of rats. A similar experiment showed HSV-CREB injections into

hippocampus improved memory for water cross maze [34]. Most recently, mice injected with HSV-CREB in the retrosplenial cortex also showed improved memory for water maze [35]. This series of experiments indicate that virally-mediated overexpression of CREB is sufficient to facilitate memory in young animals.

CREB's ability to modulate performance on behavioral tasks is unsurprising, but of particular interest are the recent studies that have reported CREB's ability to bidirectionally modulate intrinsic neuronal excitability. Expression of caCREB has resulted in increased excitability in many brain areas: CA1 hippocampus [36], locus ceruleus [37], nucleus accumbens [38], and amygdala [39]. Additionally, overexpression of endogenous CREB is sufficient to produce modest increases in excitability of neurons in the amygdala [40], [41]. This indicates there is a general mechanism activated by CREB, which works in a universal, non-region specific manner to increase intrinsic excitability of neurons. By expressing dominant negative forms of CREB, excitability can also be reduced [37], [38], [42]. Despite the evidence for CREB's ability to modulate neuronal excitability, the mechanism of how this occurs is still unknown. Many channels are thought to contribute to the post-burst AHP, including BK, SK2, and SK4. The promoter region for the BK channel encoding gene has been shown to contain binding motifs for CREB [43], suggesting that CREB can directly regulate the transcription of BK channels. However, given that increasing expression of BK channels would enlarge the post-burst AHP [44], this does not explain how CREB might enhance excitability. As for SK2 and SK4, CREB has yet to be shown to directly modulate their transcription, suggesting that their promoters may not contain binding motifs for CREB. Therefore, CREB's modulation of these channels could be either indirect, via one or more of CREB's downstream transcriptional targets, or directly by CREB via a non-transcriptional mechanism. Regardless of the mechanism itself, these studies suggest that increases in CREB activity may ameliorate age-related deficits in neuronal excitability and cognition.

CREB in aging – a therapeutic target?

Bach et al. [45] were the first to find evidence that aged mice may have deficits in CREB signaling. Aged mice were impaired in Barnes circular maze performance and *in vitro* slices had reduced LTP as compared to their young counterparts. Both of these effects were ameliorated by compounds that activated the cAMP/PKA pathway. This pathway is well known to be an upstream activator of CREB via PKA's kinase activity [14]–[17], so it's possible that the amelioration seen in this study were a result of increases in CREB activity. Biophysical experiments have demonstrated that learning-induced reductions in the AHP depend on activation of the cAMP/PKA pathway [46]–[49]. Additionally, levels of PKA itself have been found to be decreased in hippocampi of aged rats [50], which could lead to a reduction in CREB activation. Similarly, levels of CaMKIV, another kinase which phosphorylates CREB, is also reduced with age [51]. Furthermore, CREB's obligate co-activator, CREB-binding protein (CBP), is also reduced with age [52]. Age-related reductions in these crucial activators and partners of CREB could easily lead to insufficient activation of CREB in the aged hippocampus.

Reduced CREB activation is exactly what has been observed in aged animals. Young adult mice and rats exhibit increased pCREB levels following training on tasks such as inhibitory avoidance, contextual fear conditioning, cross-maze training, radial arm maze, and Morris water maze [17], [53]–[59]. Levels of pCREB have been found to be correlated with how well the animal performed on memory tests, where more pCREB is indicative of better performance [60]. In contrast, aged animals who fail to learn or remember a task also do not exhibit the learning-associated increase in pCREB levels. Specifically, immunocytochemistry studies have shown less pCREB in aged animals shortly after contextual fear conditioning [55], inhibitory avoidance [61], and water maze [59]. Western blot experiments have also revealed a reduction in total CREB in aged animals who failed to learn, when measured immediately after water maze training [62]. These results indicate that CREB levels and activity is decreased in aged brains, and suggest increasing CREB activity could ameliorate age-related deficits. Supporting this idea is a parabiosis study bringing together the circulatory systems of young and aged mice [63]. Age-related deficits in cognition and dendritic spine density in the hippocampus were reversed by young plasma, and this effect was dependent on CREB signaling.

While CREB activation in response to behavioral training has been well studied, few have examined basal levels of CREB with age. If basal levels of CREB or pCREB were indicative of cognitive status, they could be used as a biomarker. In western blot experiments on naive animals, Foster et al. [64] showed that total CREB levels are not changed, but that pCREB is decreased in hippocampus of aged (20–24 month old) Fischer 344 rats. Monti et al. [65] showed a different result; while total CREB was also found to be unchanged, pCREB was increased in aged (30 month old) Wistar rats. These studies used protein extracts from the entire hippocampus. Given that dorsal and ventral hippocampus carry out different functions, as well as the major hippocampal sub-regions (CA1, CA3, and DG), it's possible that by treating all these regions as one will mask subtle differences that may be present. One study did examine pCREB levels in aging using immunohistochemistry. Levels of pCREB were found to drop by middle age, and further decrease into old age [66]. All hippocampal sub-regions showed a decrease in pCREB: CA1 by ~25%, CA3 by ~25%, and DG by ~28%. Unfortunately, the antibody used was not specific only to pCREB, it also recognized related proteins, pCREM and pATF-1, meaning the results cannot be attributed purely to changes to pCREB. Additionally, only looking at pCREB levels do not tell us if the ratio of pCREB/CREB is changing, or if total levels of CREB are reduced. Further, experiments are needed to fully elucidate the age-related changes in basal CREB levels and activation. We recommend a full characterization of age-related changes in basal CREB levels and pCREB in each hippocampal sub-region. Furthermore, examination of CREB levels should be carried out at a time point long after the end of behavioral training to determine if a relationship exists between basal CREB and/or pCREB levels and learning and memory of the task. We expect to see either a decrease in total CREB levels, or a decrease in CREB activity, most likely in CA1 where biophysical deficits have been observed. We also expect that just like in young animals [60], pCREB levels should be related to performance on a behavioral task, most likely on memory performance.

There are also some studies that suggest that increasing CREB levels may not be beneficial. For example, levels of CREB-binding protein were not related to either chronological age, or

cognitive status in young and aged rats [67]. Optogenetic activation of A_{2A} receptors that increase levels of pCREB in the hippocampus impaired, rather than facilitated, performance on the Y-maze task [68]. Finally, age-related working memory deficit, attributed to increased pCREB levels in prelimbic cortex, were rescued by pharmacologically reducing pCREB levels [69]. The conflicting studies presented here, along with incongruous results in how basal CREB activity changes with age, necessitate further work; especially those that examine causal relationship between CREB and age-related cognitive deficits.

We propose that insufficient CREB signaling underlies age-related deficits at the biophysical and cognitive levels. If there is a causal relationship between CREB and the age-related deficits, CREB overexpression should ameliorate these deficits. These experiments could easily be carried out in Fischer 344 x Brown Norway F1 hybrid rats, which are already well-characterized to have age-related cognitive and biophysical deficits [4], [70]. To test for rescue after the onset of these deficits, as opposed to a preventative approach, viral vectors are an ideal tool. In our hands, expression of caCREB can be achieved in young rats using a viral vector. In these young rats, CA1 neurons expressing caCREB have reduced AHP amplitude (Fig. 1), replicating previous findings with caCREB expression in young mice [36]. However, caCREB must be used with caution, as expression for more than 10 or 11 days can lead to impairment on behavioral tasks [71]. To avoid the toxicity associated with caCREB, we recommend overexpression of wild-type, non-mutated CREB, which has been shown to be sufficient to increase neuronal excitability [32]–[34], [40]. Given the tight link between neuronal excitability and cognition identified by pharmacological manipulations, we expect CREB to also ameliorate age-related cognitive deficits. To detect the potential improvements in behavior with CREB overexpression, behavioral tests such as Morris water maze and trace eye blink conditioning can be used, as both of these tasks are highly sensitive to age-related cognitive deficits [4], [72], [73]. In order to achieve long-lasting overexpression of CREB through the duration of training for these tasks, we recommend using adeno-associated viruses (AAV) which have been shown to stably express for more than 15 months [74], [75]. In this proposed set of experiments, young and aged animals can be first injected with AAV into CA1 of hippocampus, and given time for virus to express. The animals would then be trained on one or more behavioral tasks to detect cognitive deficits or amelioration of such in the aged animals. Two weeks following the end of behavioral training, animals would then be euthanized to detect any changes to basal measurements of cellular excitability, such as the AHP. If dysfunctional CREB activation does indeed underlie age-related cognitive deficits, we would expect to see an amelioration of age-related cognitive deficits, most likely in the domain of long-term memory, alongside a reduction in the AHP from infected CA1 neurons.

Concluding Remarks

CREB is one of the most well studied proteins that are important for memory formation in young adult animals. Other proteins, such as early growth response protein 1 (EGR-1) and brain-derived neurotrophic factor (BDNF), have also been demonstrated to be important for memory formation in young adult animals. However, like CREB, much is yet to be discovered about the aging-related changes in their function and/or activity. Moreover, it is possible that there is an interaction of those proteins, such as CREB and BDNF, in a

signaling cascade that ultimately leads to successful memory formation. Hence, altering the activity of only CREB may not fully rescue the aging-related cognitive deficits. However, given the impact of altering just the CREB levels on memory formation in young adults, we hypothesize that increasing CREB activity will ameliorate the cognitive-deficits observed in normal aged subjects.

Acknowledgments

This work was supported by NIH grants R37 AG008796 (JFD), RF1 AG017139 (JFD), T32 AG020506 (XY) and the Glenn/AFAR Scholarship for Research in the Biology of Aging (XY). These funding sources had no involvement in the design of this study; the collection, analysis, or interpretation of the data; or in the writing of this report and where to submit for publication. The authors thank Dr. Angel Barco for providing the caCREB construct, and the Northwestern University's DNA/RNA Delivery Core for lentiviral production.

Abbreviations

AHP	afterhyperpolarization
BDNF	brain-derived neurotrophic factor
caCREB	constitutively-active CREB
CRE	cAMP response element
CREB	cAMP response element binding-protein
EGR-1	early growth response protein 1
LTP	long-term potentiation

References

1. Barnes CA. Memory deficits associated with senescence: A neurophysiological and behavioral study in the rat. *Journal of Comparative and Physiological Psychology*. 1979; 93(1):74–104. [PubMed: 221551]
2. Foster TC. Involvement of hippocampal synaptic plasticity in age-related memory decline. *Brain Research Reviews*. Nov; 1999 30(3):236–249. [PubMed: 10567726]
3. Thompson LT, Moyer JR, Disterhoft JF. Trace eyeblink conditioning in rabbits demonstrates heterogeneity of learning ability both between and within age groups. *Neurobiol Aging*. Jul; 1996 17(4):619–629. [PubMed: 8832637]
4. Knutinen MG, Gamelli AE, Weiss C, Power JM, Disterhoft JF. Age-related effects on eyeblink conditioning in the F344 x BN F1 hybrid rat. *Neurobiol Aging*. Jan; 2001 22(1):1–8. [PubMed: 11164270]
5. Disterhoft JF, Oh MM. Learning, aging and intrinsic neuronal plasticity. *Trends Neurosci*. Oct; 2006 29(10):587–599. [PubMed: 16942805]
6. Disterhoft JF, Oh MM. Alterations in intrinsic neuronal excitability during normal aging. *Aging Cell*. Jun; 2007 6(3):327–336. [PubMed: 17517042]
7. Landfield PW, McGaugh JL, Lynch G. Impaired synaptic potentiation processes in the hippocampus of aged, memory-deficient rats. *Brain Research*. Jul; 1978 150(1):85–101. [PubMed: 208716]
8. Oh MM, Oliveira FA, Disterhoft JF. Learning and aging related changes in intrinsic neuronal excitability. *Front Aging Neurosci*. 2010; 2:2–2. [PubMed: 20552042]
9. Landfield PW, Pitler TA. Prolonged Ca²⁺-dependent afterhyperpolarizations in hippocampal neurons of aged rats. *Science*. Sep.1984 226:1089–1092. [PubMed: 6494926]

10. Moyer JR, Thompson LT, Black JP, Disterhoft JF. Nimodipine increases excitability of rabbit CA1 pyramidal neurons in an age- and concentration-dependent manner. *Journal of Neurophysiology*. Dec; 1992 68(6):2100–2109. [PubMed: 1491260]
11. Deyo RA, Straube KT, Disterhoft JF. Nimodipine facilitates associative learning in aging rabbits. *Science*. Feb; 1989 243(4892):809–811. [PubMed: 2916127]
12. Weible AP, Oh MM, Lee G, Disterhoft JF. Galantamine Facilitates Acquisition of Hippocampus-Dependent Trace Eyeblink Conditioning in Aged Rabbits. *Learning & Memory*. Jan; 2004 11(1):108–115. [PubMed: 14747524]
13. Disterhoft JF, Moyer JR, Thompson LT. The calcium rationale in aging and Alzheimer's disease. Evidence from an animal model of normal aging. *Ann N Y Acad Sci*. Dec.1994 747:382–406. [PubMed: 7847686]
14. Deisseroth K, Heist EK, Tsien RW. Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. *Nature*. Mar; 1998 392(6672):198–202. [PubMed: 9515967]
15. Dash PK, Hochner B, Kandel ER. Injection of the Camp-Responsive Element Into the Nucleus of Aplysia Sensory Neurons Blocks Long-Term Facilitation. *Nature*. 1990; 345(6277):718–721. [PubMed: 2141668]
16. Kaang BK, Kandel ER, Grant SGN. Activation of cAMP-Responsive genes by stimuli that produce long-term facilitation in aplysia sensory neurons. *Neuron*. Mar; 1993 10(3):427–435. [PubMed: 8384857]
17. Bernabeu R, Bevilacqua L, Ardenghi P, Bromberg E, Schmitz P, Bianchin M, Izquierdo I, Medina JH. Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. *Proceedings of the National Academy of Sciences of the United States of America*. Jun; 1997 94(13):7041–7046. [PubMed: 9192688]
18. Alberini CM. Transcription factors in long-term memory and synaptic plasticity. *Physiol Rev*. Jan; 2009 89(1):121–145. [PubMed: 19126756]
19. Impey S, Smith DM, Obrietan K, Donahue R, Wade C, Storm DR. Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nat Neurosci*. Nov; 1998 1(7):595–601. [PubMed: 10196567]
20. Guzowski JFJ, McGaugh JLJ. Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. *Proceedings of the National Academy of Sciences of the United States of America*. Mar; 1997 94(6):2693–2698. [PubMed: 9122258]
21. Bourchouladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G, Silva AJ. Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell*. Oct; 1994 79(1):59–68. [PubMed: 7923378]
22. Barco A, Pittenger C, Kandel ER. CREB, memory enhancement and the treatment of memory disorders: promises, pitfalls and prospects. *Expert Opin Ther Targets*. Feb; 2003 7(1):101–114. [PubMed: 12556206]
23. Tully T, Bourchouladze R, Scott R, Tallman J. Targeting the CREB pathway for memory enhancers. *Nat Rev Drug Discov*. Apr; 2003 2(4):267–277. [PubMed: 12669026]
24. Johannessen M, Delghandi MP, Moens U. What turns CREB on? *Cellular Signalling*. Nov; 2004 16(11):1211–1227. [PubMed: 15337521]
25. Carlezon WA, Duman R, Nestler EJ. The many faces of CREB. *Trends Neurosci*. Aug; 2005 28(8):436–445. [PubMed: 15982754]
26. Pittenger C, Huang YY, Paletzki RF, Bourchouladze R, Scanlin H, Vronskaya S, Kandel ER. Reversible Inhibition of CREB/ATF Transcription Factors in Region CA1 of the Dorsal Hippocampus Disrupts Hippocampus-Dependent Spatial Memory. *Neuron*. Apr; 2002 34(3):447–462. [PubMed: 11988175]
27. Kida SS, Josselyn SAS, de Ortiz SSP, Kogan JHJ, Chevere II, Masushige SS, Silva AJA. CREB required for the stability of new and reactivated fear memories. *Nat Neurosci*. Apr; 2002 5(4):348–355. [PubMed: 11889468]

28. Monti B, Berteotti C, Contestabile A. Subchronic Rolipram Delivery Activates Hippocampal CREB and Arc, Enhances Retention and Slows Down Extinction of Conditioned Fear. *Neuropsychopharmacology*. Jun; 2005 31(2):278–286.
29. Suzuki A, Fukushima H, Mukawa T, Toyoda H, Wu LJ, Zhao MG, Xu H, Shang Y, Endoh K, Iwamoto T, Mamiya N, Okano E, Hasegawa S, Mercaldo V, Zhang Y, Maeda R, Ohta M, Josselyn SA, Zhuo M, Kida S. Upregulation of CREB-mediated transcription enhances both short- and long-term memory. *The Journal of Neuroscience*. Jun; 2011 31(24):8786–8802. [PubMed: 21677163]
30. Viosca J, de Armentia ML, Jancic D, Barco A. Enhanced CREB-dependent gene expression increases the excitability of neurons in the basal amygdala and primes the consolidation of contextual and cued fear memory. *Learning & Memory*. Feb; 2009 16(3):193–197. [PubMed: 19237641]
31. Restivo L, Tafi E, Ammassari-Teule M, Marie H. Viral-mediated expression of a constitutively active form of CREB in hippocampal neurons increases memory. *Hippocampus*. Mar; 2009 19(3):228–234. [PubMed: 19004015]
32. Sekeres MJ, Neve RL, Frankland PW, Josselyn SA. Dorsal hippocampal CREB is both necessary and sufficient for spatial memory. *Learn Mem*. Jun; 2010 17(6):280–283. [PubMed: 20495061]
33. Josselyn SA, Shi C, Carlezon WA, Neve RL, Nestler EJ, Davis M. Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala. *The Journal of Neuroscience*. Apr; 2001 21(7):2404–2412. [PubMed: 11264314]
34. Brightwell JJ, Smith CA, Neve RL, Colombo PJ. Long-term memory for place learning is facilitated by expression of cAMP response element-binding protein in the dorsal hippocampus. *Learning & Memory*. Mar; 2007 14(3):195–199. [PubMed: 17351144]
35. Czajkowski, R., Jayaprakash, B., Wiltgen, B., Rogerson, T., Guzman-Karlsson, MC., Barth, AL., Trachtenberg, JT., Silva, AJ. Encoding and storage of spatial information in the retrosplenial cortex. *Proceedings of the National Academy of Sciences*; May 2014;
36. Lopez de Armentia M, Jancic D, Olivares R, Alarcon JM, Kandel ER, Barco A. cAMP response element-binding protein-mediated gene expression increases the intrinsic excitability of CA1 pyramidal neurons. *Journal of Neuroscience*. 2007; 27(50):13909–13918. [PubMed: 18077703]
37. Han MH, Bolaños CA, Green TA, Olson VG, Neve RL, Liu RJ, Aghajanian GK, Nestler EJ. Role of cAMP response element-binding protein in the rat locus ceruleus: regulation of neuronal activity and opiate withdrawal behaviors. *Journal of Neuroscience*. Apr; 2006 26(17):4624–4629. [PubMed: 16641242]
38. Dong Y, Green T, Saal D, Marie H, Neve R, Nestler EJ, Malenka RC. CREB modulates excitability of nucleus accumbens neurons. *Nat Neurosci*. Mar; 2006 9(4):475–477. [PubMed: 16520736]
39. Viosca J, Malleret G, Bourchouladze R, Benito E, Vronskava S, Kandel ER, Barco A. Chronic enhancement of CREB activity in the hippocampus interferes with the retrieval of spatial information. *Learning & Memory*. Feb; 2009 16(3):198–209. [PubMed: 19237642]
40. Zhou Y, Won J, Karlsson MG, Zhou M, Rogerson T, Balaji J, Neve R, Poirazi P, Silva AJ. CREB regulates excitability and the allocation of memory to subsets of neurons in the amygdala. *Nat Neurosci*. Sep; 2009 12(11):1438–1443. [PubMed: 19783993]
41. Yiu AP, Mercaldo V, Yan C, Richards B, Rashid AJ, Hsiang HLL, Pressey J, Mahadevan V, Tran MM, Kushner SA, Woodin MA, Frankland PW, Josselyn SA. Neurons Are Recruited to a Memory Trace Based on Relative Neuronal Excitability Immediately before Training. *Neuron*. Aug; 2014 83(3):722–735. [PubMed: 25102562]
42. Jancic D, Lopez de Armentia M, Valor LM, Olivares R, Barco A. Inhibition of cAMP Response Element-Binding Protein Reduces Neuronal Excitability and Plasticity, and Triggers Neurodegeneration. *Cerebral Cortex*. Oct; 2009 19(11):2535–2547. [PubMed: 19213815]
43. Wang Y, Ghezzi A, Yin JCP, Atkinson NS. CREB regulation of BK channel gene expression underlies rapid drug tolerance. *Genes Brain and Behavior*. Jun; 2009 8(4):369–376.
44. McKay BM, Oh MM, Galvez R, Burgdorf J, Kroes RA, Weiss C, Adelman JP, Moskal JR, Disterhoft JF. Increasing SK2 channel activity impairs associative learning. *Journal of Neurophysiology*. Aug; 2012 108(3):863–870. [PubMed: 22552186]

45. Bach MEM, Barad MM, Son HH, Zhuo MM, Lu YFY, Shih RR, Mansuy II, Hawkins RDR, Kandel ERE. Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proceedings of the National Academy of Sciences of the United States of America*. Apr; 1999 96(9):5280–5285. [PubMed: 10220457]
46. Pedarzani P, Storm JF. Dopamine modulates the slow Ca(2+)-activated K⁺ current IAHP via cyclic AMP-dependent protein kinase in hippocampal neurons. *Journal of Neurophysiology*. Dec; 1995 74(6):2749–2753. [PubMed: 8747230]
47. Pedarzani P, Storm JF. PKA mediates the effects of monoamine transmitters on the K⁺ current underlying the slow spike frequency adaptation in hippocampal neurons. *Neuron*. Dec; 1993 11(6):1023–1035. [PubMed: 8274274]
48. Oh MM, McKay BM, Power JM, Disterhoft JF. Learning-related postburst afterhyperpolarization reduction in CA1 pyramidal neurons is mediated by protein kinase A. *Proceedings of the National Academy of Sciences*. Feb; 2009 106(5):1620–1625.
49. Zhang L, Ouyang M, Ganellin CR, Thomas SA. The slow afterhyperpolarization: a target of β 1-adrenergic signaling in hippocampus-dependent memory retrieval. *The Journal of Neuroscience*. Mar; 2013 33(11):5006–5016. [PubMed: 23486971]
50. Karege F, Schwald M, Lamercy C, Murama JJ, Cisse M, Malafosse A. A non-radioactive assay for the cAMP-dependent protein kinase activity in rat brain homogenates and age-related changes in hippocampus and cortex. *Brain Research*. Jun; 2001 903(1):86–93. [PubMed: 11382391]
51. Fukushima H, Maeda R, Suzuki R, Suzuki A, Nomoto M, Toyoda H, Wu L-J, Xu H, Zhao M-G, Ueda K, Kitamoto A, Mamiya N, Yoshida T, Homma S, Masushige S, Zhuo M, Kida S. Upregulation of calcium/calmodulin-dependent protein kinase IV improves memory formation and rescues memory loss with aging. *The Journal of Neuroscience*. Oct; 2008 28(40):9910–9919. [PubMed: 18829949]
52. Chung YH, Kim EJ, Shin CM, Joo KM, Kim MJ, Woo HW, Cha CI. Age-related changes in CREB binding protein immunoreactivity in the cerebral cortex and hippocampus of rats. *Brain Research*. Nov; 2002 956(2):312–318. [PubMed: 12445700]
53. Taubenfeld SM, Wiig KA, Monti B, Dolan B, Pollonini G, Alberini CM. Fornix-dependent induction of hippocampal CCAAT enhancer-binding protein [beta] and [delta] Co-localizes with phosphorylated cAMP response element-binding protein and accompanies long-term memory consolidation. *The Journal of Neuroscience*. Jan; 2001 21(1):84–91. [PubMed: 11150323]
54. Stanciu M, Radulovic J, Spiess J. Phosphorylated cAMP response element binding protein in the mouse brain after fear conditioning: relationship to Fos production. *Brain Res Mol Brain Res*. Oct; 2001 94(1):15–24. [PubMed: 11597761]
55. Kudo K, Wati H, Qiao C, Arita J, Kanba S. Age-related disturbance of memory and CREB phosphorylation in CA1 area of hippocampus of rats. *Brain Research*. Aug; 2005 1054(1):30–37. [PubMed: 16054117]
56. Colombo PJP, Brightwell JJJ, Countryman RAR. Cognitive strategy-specific increases in phosphorylated cAMP response element-binding protein and c-Fos in the hippocampus and dorsal striatum. *CORD Conference Proceedings*. Apr; 2003 23(8):3547–3554.
57. Mizuno MM, Yamada KK, Maekawa NN, Saito KK, Seishima MM, Nabeshima TT. CREB phosphorylation as a molecular marker of memory processing in the hippocampus for spatial learning. *Behav Brain Res*. Jul; 2002 133(2):135–141. [PubMed: 12110446]
58. Porte Y, Buhot MC, Mons NE. Spatial memory in the Morris water maze and activation of cyclic AMP response element-binding (CREB) protein within the mouse hippocampus. *Learning & Memory*. Dec; 2008 15(12):885–894. [PubMed: 19050160]
59. Porte Y, Buhot MC, Mons N. Alteration of CREB phosphorylation and spatial memory deficits in aged 129T2/Sv mice. *Neurobiol Aging*. Oct; 2008 29(10):1533–1546. [PubMed: 17478013]
60. Cowansage KK, Bush DEA, Josselyn SA, Klann E, Ledoux JE. Basal variability in CREB phosphorylation predicts trait-like differences in amygdala-dependent memory. *Proceedings of the National Academy of Sciences*. Oct; 2013 110(41):16645–16650.

61. Morris KA, Gold PE. Age-related impairments in memory and in CREB and pCREB expression in hippocampus and amygdala following inhibitory avoidance training. *Mech Ageing Dev.* May; 2012 133(5):291–299. [PubMed: 22445851]
62. Brightwell JJ, Gallagher M, Colombo PJ. Hippocampal CREB1 but not CREB2 is decreased in aged rats with spatial memory impairments. *Neurobiology of Learning and Memory.* Jan; 2004 81(1):19–26. [PubMed: 14670355]
63. Villeda SA, Plambeck KE, Middeldorp J, Castellano JM, Mosher KI, Luo J, Smith LK, Bieri G, Lin K, Berdnik D, Wabl R, Udeochu J, Wheatley EG, Zou B, Simmons DA, Xie XS, Longo FM, Wyss-Coray T. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nat Med.* May; 2014 20(6):659–663. [PubMed: 24793238]
64. Foster TC, Sharrow KM, Masse JR, Norris CM, Kumar A. Calcineurin links Ca²⁺ dysregulation with brain aging. *The Journal of Neuroscience.* Jun; 2001 21(11):4066–4073. [PubMed: 11356894]
65. Monti B, Berteotti C, Contestabile A. Dysregulation of memory-related proteins in the hippocampus of aged rats and their relation with cognitive impairment. *Hippocampus.* 2005; 15(8):1041–1049. [PubMed: 16086428]
66. Hattiangady B, Rao MS, Shetty GA, Shetty AK. Brain-derived neurotrophic factor, phosphorylated cyclic AMP response element binding protein and neuropeptide Y decline as early as middle age in the dentate gyrus and CA1 and CA3 subfields of the hippocampus. *Exp Neurol.* Oct; 2005 195(2):353–371. [PubMed: 16002067]
67. Pereira IT, Coletta CE, Perez EV, Kim DH. CREB-binding protein levels in the rat hippocampus fail to predict chronological or cognitive aging. ... of Aging. 2012
68. Li P, Rial D, Canas PM, Yoo J-H, Li W, Zhou X, Wang Y, van Westen GJP, Payen M-P, Augusto E, Gonçalves N, Tomé AR, Li Z, Wu Z, Hou X, Zhou Y, IJzerman AP, PIJzerman A, Boyden ES, Cunha RA, Qu J, Chen J-F. Optogenetic activation of intracellular adenosine A2A receptor signaling in the hippocampus is sufficient to trigger CREB phosphorylation and impair memory. *Mol Psychiatry.* Nov; 2015 20(11):1339–1349. [PubMed: 25687775]
69. Vandesquille M, Baudonnat M, Decorte L, Louis C, Lestage P, Béracochéa D. Working memory deficits and related disinhibition of the cAMP/PKA/CREB are alleviated by prefrontal $\alpha 4\beta 2^*$ -nAChRs stimulation in aged mice. *Neurobiol Aging.* Jun; 2013 34(6):1599–1609. [PubMed: 23352115]
70. Matthews EA, Linardakis JM, Disterhoft JF. The Fast and Slow Afterhyperpolarizations Are Differentially Modulated in Hippocampal Neurons by Aging and Learning. *Journal of Neuroscience.* Apr; 2009 29(15):4750–4755. [PubMed: 19369544]
71. Gruart A, Benito E, Delgado-Garcia JM, Barco A. Enhanced cAMP Response Element-Binding Protein Activity Increases Neuronal Excitability, Hippocampal Long-Term Potentiation, and Classical Eyeblick Conditioning in Alert Behaving Mice. *The Journal of Neuroscience.* Nov; 2012 32(48):17431–17441. [PubMed: 23197734]
72. Gallagher M, Nicolle MM. Animal models of normal aging: Relationship between cognitive decline and markers in hippocampal circuitry. *Behav Brain Res.* Nov; 1993 57(2):155–162. [PubMed: 7906946]
73. Foster TC. Dissecting the age-related decline on spatial learning and memory tasks in rodent models: N-methyl-D-aspartate receptors and voltage-dependent Ca²⁺ channels in senescent synaptic plasticity. *Prog Neurobiol.* Mar; 2012 96(3):283–303. [PubMed: 22307057]
74. Mouravlev A, Dunning J, Young D, During MJ. Somatic gene transfer of cAMP response element-binding protein attenuates memory impairment in aging rats. *Proceedings of the National Academy of Sciences of the United States of America.* Mar; 2006 103(12):4705–4710. [PubMed: 16537429]
75. Klein RL, Hamby ME, Gong Y, Hirko AC, Wang S, Hughes JA, King MA, Meyer EM. Dose and promoter effects of adeno-associated viral vector for green fluorescent protein expression in the rat brain. *Exp Neurol.* Jul; 2002 176(1):66–74. [PubMed: 12093083]

Highlights

- Biophysical and cognitive deficits are linked in aging
- CREB modulates biophysics and cognition in young adult animals
- CREB dysfunction may underlie biophysical and cognitive changes in aging
- CREB may be a therapeutic target for age-related cognitive deficits

Figure 1.

Constitutively-active CREB expression reduces peak and slow AHP evoked with 15 suprathreshold current injections at 50Hz in CA1 pyramidal neurons from young adult rats. Whole-cell current clamp recording experiments were conducted 2 weeks after the injection of a lentiviral vector to express VP16-CREB (caCREB) in CA1 of the hippocampus. The caCREB sequence was downstream from a hSyn promoter, followed by an EGFP reporter (downstream of its own hSyn promoter), to allow for visualization of infected cells. **a**) Example stitched 10x confocal image of EGFP expression 2 weeks post-injection of lentivirus into dorsal CA1 area. Summed z-stack of infected CA1 pyramidal neurons (inset). Left scale bar 250 μm , right scale bar 100 μm . **b**) Peak AHP amplitudes measured from caCREB-infected cells (green: $n = 11$) are smaller than that of neighboring uninfected

control cells (blue: $n = 10$) (unpaired t-test: $* p < 0.05$). c) Slow AHP amplitudes measured from caCREB-infected cells are also smaller than that of neighboring uninfected control cells ($** p < 0.01$). d) Representative traces from AHP recordings made from caCREB-infected and control cells, arrow indicates 1 s time point where slow AHP was measured. Bars in b and c represent mean $\pm$ S.E.M.