Psych 3102
Introduction to Behavioral Genetics

Lecture 18

Cognitive disabilities continued:

Dementia

Dementia

- severe cognitive decline

- age related: <1% under 65
  13% of those aged 65
  42% of those aged 85+

US Medicare cost/year $189 billion by 2015
not including cost to family, friends, insurance

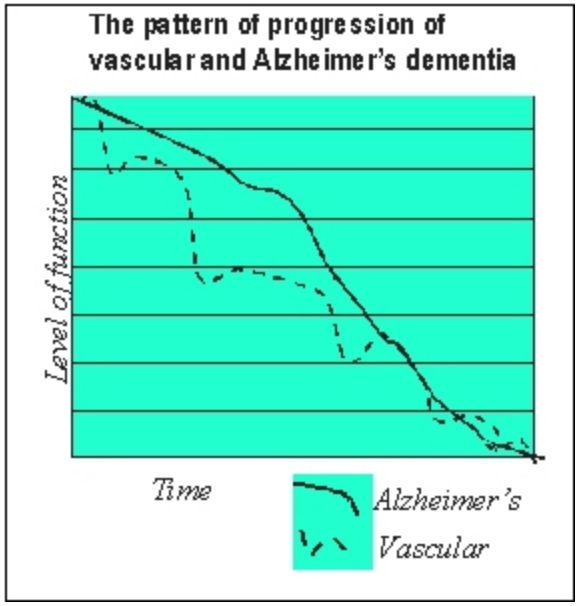
2nd leading cause of death
2nd most expensive medical problem amongst elderly

by 2050 US 1 in 30 will have dementia
will bankrupt Medicaid/Medicare if no treatment is discovered

Types of dementia

Multiple infarct dementia (MID)(vascular dementia)

- numerous small strokes
- infarcts lead to brain damage
- more abrupt onset, focal symptoms



Alzheimers disease (AD)

- half of all dementia cases

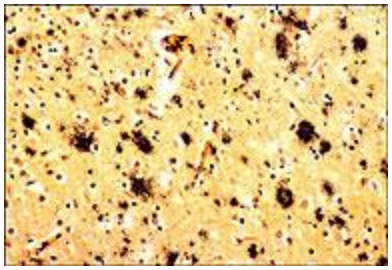
1/3 of all dementia cases involve both AD and MID

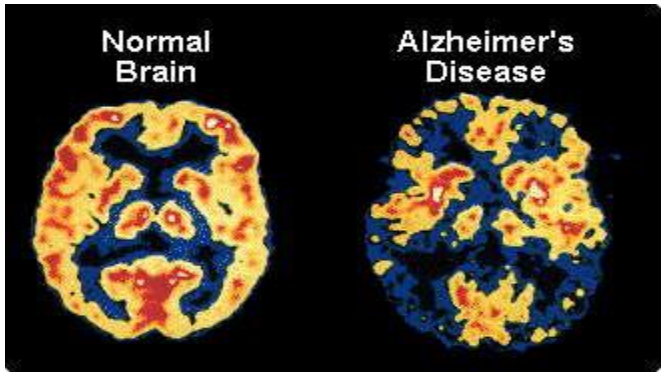
Other: Pick's disease, frontotemporal lobe dementia

- all except MID seem to involve tau gene, chr 17
- chronic inflammation is a feature of aging brain and may play important role in several neurodegenerative diseases

astrocytes normally suppress inflammation through activation of DRD2 receptors – new avenue of research into treatments

Alzheimer's disease (AD)





- 50% of all dementia cases
- of top 10 causes of death, AD is the only one with increasing mortality
- early and late-onset types

-symptoms very similar but progression swifter in early onset, same eventual outcome

Disease progression: recent memory loss, irritability, poor concentration
loss of independence

death usually within 4-6 years after diagnosis for late onset (range 3-20 years)

- once symptoms appear extensive changes in the brain have already occurred extensive amyloid peptide buildup, tangles of fibers, plaques
- disease thought to start much earlier than current diagnosis

AD

no effective treatment

current ‘treatments’: 5 drugs slow progress for 6-12 months in ½ of those treated

no proven means of prevention

definitive diagnosis used to be on autopsy

now, brain imaging, spinal tap 90% accurate

spinal tap reveals β amyloid elevated 20-30 yrs before onset of symptoms ie. at 10,20 yrs of age in early onset cases

late onset APOE4 carriers – brain function changes measured at 40 yrs of age, before any amyloid changes

failure of new treatments thought to be because disease needs to be treated *prior* to symptom onset

~ 58% heritability for late onset type (early onset is single gene)

genes have slightly greater influence than environment

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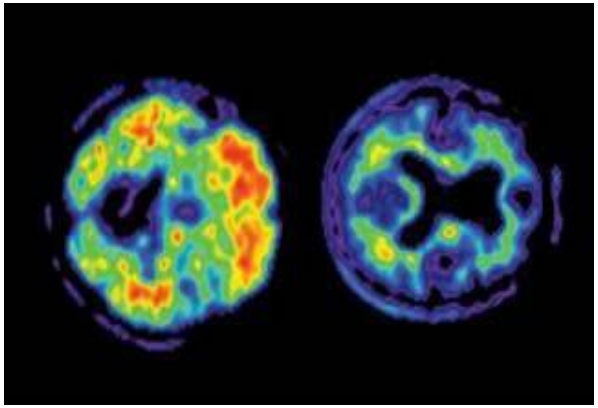
News

Alzheimer's-disease probe nears approval

Imaging technique could help to resolve questions about brain plaques associated with the condition.

Heidi Ledford



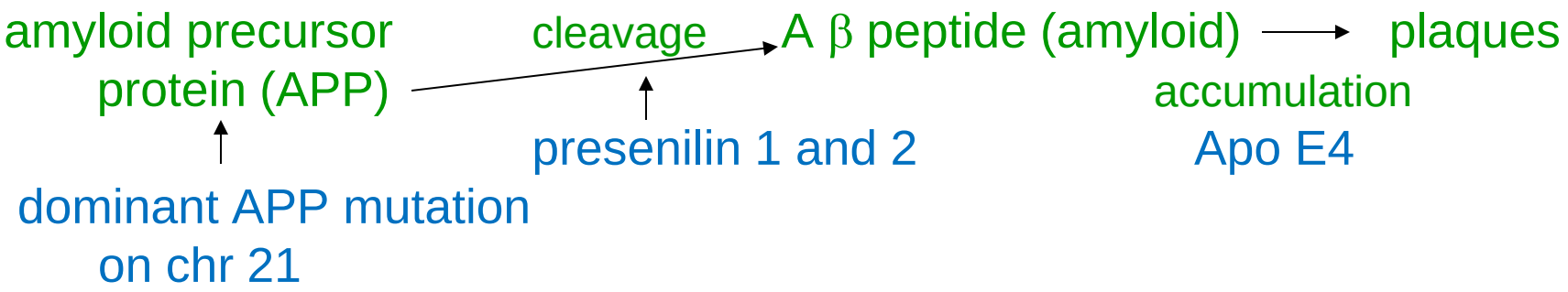
Flor betapir reveals amyloid plaque build-up (red) in the brain of someone with Alzheimer's disease (bottom), which is absent in a healthy brain (top).*ELI Lilly/Avid Radiopharmaceuticals*

An imaging agent that reveals a signature of Alzheimer's disease in the brain — given conditional support last week by advisers to the US Food and Drug Administration (FDA) — is likely to be more valuable to scientists than to patients. The agent, called florbetapir (Amyvid), enables physicians to determine whether Alzheimer's disease is the cause of a patient's dementia. In the future, it may also help them to catch the disease before obvious symptoms appear, a hope that has sparked fresh debate about the value of early diagnosis for a devastating, untreatable disease. The panel of advisers — whose guidance is usually, but not always, followed by the FDA — also stated that the test should not be given final approval until its developers demonstrate that clinicians can uniformly interpret its results. "The importance of the decision is probably bigger for research in the near future than it is for clinical practice," says William Thies, the chief medical and scientific officer of the Alzheimer's Association based in Chicago, Illinois, a nonprofit organization that funds research on Alzheimer's disease. Physicians diagnose Alzheimer's disease only after memory loss interferes with daily activities. By then, "there's so much irreversible damage that it might be too late to hope for an effective treatment", says Gil Rabinovici, a neurologist at the University of California, San Francisco. Definitive confirmation comes from autopsy, with the presence of characteristic lesions in the brain caused by clumps of the peptide amyloid-β. These amyloid plaques are hypothesized to be the cause of the memory loss. Some researchers already use a reagent called Pittsburgh Compound B to image amyloid plaques in people suspected to have Alzheimer's disease. The compound binds to the plaques, and its radioactivity can be detected using positron emission tomography. But the reagent is labelled with carbon-11: with a half-life of just 20 minutes, its use is limited to the handful of facilities that have an on-site cyclotron to prepare it. In contrast, florbetapir is labelled with fluorine-18

Early-onset form

- only 10% of Alzheimers cases are early onset
- runs in families in a Mendelian way so clearly influenced by single genes
- several single gene autosomal dominant mutations found
- those with mutations will get AD, usually around 30,40 years of age
- involves over-production of β amyloid

plaques = areas of insoluble beta amyloid peptide
do not necessarily indicate areas of neuron damage
no agreement on how, or even if, amyloid is toxic



Late-onset form

- first degree relative risk = 50% (by age 85)
- prevalence = 42%

- twin studies indicate a moderate genetic influence:
  MZ concordance = 60%
  DZ concordance = 30%
  h^2 estimated 58%

- thought to be caused by failure to clear β amyloid

Etiology?

βamyloid binds to normal prion protein - in hippocampus this seems to be involved in LTP → learning & memory abnormal Aβ disrupts this?

APP binds to apoptosis-inducing protein DR6(death receptor 6) to regulate natural degeneration (pruning) of neurons during development

in later life, extracellular fragment of APP, working with DR6 and caspase6 produces neurodegeneration?

Apolipoprotein E gene chromosome 19

- associated with nearly 50% of late-onset AD cases
- typical association: E4 allele found in 40% cases compared to 15% controls

3 alleles :

E2 rare allele, protective effect on AD (but homozygotes have > risk for cv disease via high blood cholesterol)

E3 most frequent allele

E4 risk allele increases AD x6 (also increases atherosclerosis → heart attack stroke)

Genotype	**Frequency**	**Mean age of onset**	**Risk of AD**
E4/E4	2% population	68	1 in 2
E4/ -	15%	75	1 in 4
no E4	83%	84	1 in 10

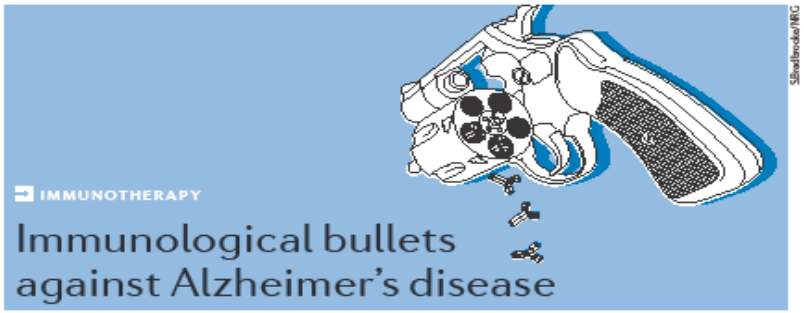
TREM2 gene chromosome 6

- recently reported (Jonsson et al, NEJM, Nov 1012), independent replication reported in GWAS study
- rare missense mutation
- OR = 2.92
- receptor on microglia, dendrites – role in inflammation

risk allele thought to cause brain damage through inability to clear toxic products

- mouse knock-out – reduced phagocytosis of apoptotic neurons
  overexpression increases phagocytosis

Several other rare, lower risk variants also identified by other GWAS that also influence inflammation



IMMUNOTHERAPY

Immunological bullets against Alzheimer's disease

Alzheimer's disease is associated with the increased production and accumulation of amyloid-β peptides in the brain. An inflammatory component has also been recognized, although the exact contribution of innate immune signalling to disease pathogenesis is still unclear. Here, vom Berg, Prokop and colleagues identify interleukin-12 (IL-12) and IL-23 signalling as a therapeutic target in this neurodegenerative disorder.

Microglia are brain- and spinal cord-resident cells of myeloid origin, and there has been some debate regarding whether they contribute to the clearance of amyloid-β following their activation. The authors analysed the phenotype of microglia from APP/PS1 transgenic mice, which are used as a model of Alzheimer's disease, and found increased expression of p40 (the common subunit of IL-12 and IL-23) compared with microglia from wild-type mice. So, they crossed APP/PS1 mice with mice that are deficient for p40, p35 (the α-subunit of IL-12) or p19 (the α-subunit of IL-23) to investigate the role of these cytokines in Alzheimer's disease pathogenesis.

> "microglial cell-derived IL-12 and IL-23 ... are involved in disease progression"

Strikingly, these cytokine-knockout APP/PS1 mice showed reduced disease severity compared with APP/PS1 mice, as assessed by the accumulation of amyloid-β in young mice and the formation of amyloid-β plaques in older mice. Moreover, experiments with bone marrow-chimeric mice indicated that microglial cell-derived IL-12 and IL-23 (but not peripheral myeloid cell-derived IL-12 and IL-23) are involved in disease progression.

So, can these findings have a therapeutic application? Intraperitoneal administration of a p40-specific antibody for 11–13 weeks reduced early amyloid-β plaque formation (at 120 days of age) in APP/PS1 mice. Moreover, intracerebroventricular administration of the p40-specific antibody in older APP/PS1 mice ameliorated some behavioural and cognitive deficits. In addition, the concentration of p40 in the cerebrospinal fluid of patients with Alzheimer's disease was shown to correlate with the patients' performance in mental evaluation tests, supporting a role for IL-12 and IL-23 in human disease. These results are promising from a translational perspective given that the safety of p40-specific antibodies has been previously evaluated in clinical trials for the treatment of psoriasis, Crohn's disease and multiple sclerosis.

Finally, gene expression analyses by the authors and others suggest that microglial cell-derived IL-12 and IL-23 may act by modulating the activity of astrocytes, which express the signalling subunit of the IL-12 receptor and the IL-23 receptor. However, further studies are needed to fully understand how IL-12 and IL-23 contribute to Alzheimer's disease pathogenesis.

Maria Papatriantafyllou,
Associate Editor,
Nature Reviews Immunology

This article originally appeared in *Nature Rev. Immunol.* (doi:10.1038/nri3568).

ORIGINAL RESEARCH PAPER vom Berg, J. *et al.* Inhibition of IL-12/IL-23 signaling reduces Alzheimer's disease-like pathology and cognitive decline. *Nature Med.* 25 Nov 2012 (doi:10.1038/nm.2965)

Recurrent fusion in pediatric AMKL

James Downing and colleagues report the discovery of recurrent driver mutations in non-Down syndrome acute megakaryoblastic leukemia (non-DS-AMKL), including a *CBFA2T3-GLIS2* fusion present in 27% of pediatric cases (*Cancer Cell* 22, 683–697, 2012). The authors sequenced the transcriptomes of 14 pediatric AMKL cases and found 7 with a balanced inversion on chromosome 16 resulting in an in-frame fusion of *CBFA2T3* and *GLIS2*. Follow-up analyses in a larger set of pediatric and adult AMKL cases showed that this fusion was recurrent, restricted to pediatric cases and associated with unfavorable outcome. The authors also identified a *NUP98-KDM5A* fusion in 8% of pediatric cases, as well as recurrent mutations in *GATA1* and JAK kinase genes. To examine the biological effects of the *CBFA2T3-GLIS2* fusion, the authors transduced mouse hematopoietic cells with a retrovirus encoding *CBFA2T3-GLIS2* and found that the fusion conferred increased capacity for self-renewal, with evidence for differentiation along the megakaryocytic lineage. They further showed that these effects on self-renewal were likely mediated by upregulation of bone morphogenetic protein (BMP) signaling. These findings provide insights into the biology of AMKL and identify *CBFA2T3-GLIS2* as a new clinical marker with prognostic significance for pediatric patients with AMKL. ***KV***

TREM2 and Alzheimer's disease

Two groups have independently identified a rare variant that is associated with susceptibility to late-onset Alzheimer's disease (LOAD), with an effect size similar to that of the ε4 risk allele of apolipoprotein E. Researchers in the Alzheimer Genetic Analysis Group (*N. Engl. J. Med.*, published online 14 November 2012; doi:10.1056/NEJMoa1211851) nominated *TREM2* as a promising candidate because homozygous loss-of-function *TREM2* mutations cause Nasu-Hakola disease, a rare recessive, early-onset form of dementia with leukoencephalopathy and bone cysts. They generated exome sequence data sets and identified a TREM2 missense variant, R47H, which associated with LOAD in cohorts from North America and Europe. Separately, researchers at deCODE Genetics (*N. Engl. J. Med.*, published online 14 November 2012; doi:10.1056/NEJMoa1211103) identified the R47H variant in an imputation-based genome-wide association study using the Icelandic population and replicated the association with LOAD in North American and European cohorts. TREM2 is an immune phagocytic receptor expressed in brain microglia. Assuming that the *TREM2* risk variants impair TREM2 function, these studies suggest that reduced function of TREM2 causes reduced phagocytic clearance of amyloid proteins or cellular debris and thus impairs a protective mechanism in the brain. ***EN***

Migration from trees to graphs

Joseph Pickrell and Jonathan Pritchard report a new statistical model for demographic inference from genome-wide allele frequency data sets (*PLoS Genet*, 8, e1002967, 2012). Implemented in the freely available software TreeMix (http://treemix.googlecode.com/), their approach involves building a population tree, finding populations that show poor fit to the tree model and modeling migration events to improve the fit within a graph-based model. They tested their method in simulations as well as on a human data set including genome-wide data from 53 modern and 2 archaic populations. They inferred a maximum-likelihood tree from the human data set that recapitulates known population relationships and explains 98.8% of the variance in relatedness between populations. By sequentially adding the ten migration events that are most consistent with known events, they increased the explained variance to 99.8%. The authors also applied their approach to a canine genome-wide data set of 82 dog breeds or wild canids. They found that a significant amount of gene flow occurred between breeds during dog domestication and again demonstrate the ability to increase the explained variance in relatedness between dog breeds by sequentially adding migration events. For both humans and dogs, the authors infer many migration events, including previously known and new relationships between populations. ***OB***

MED12 in cancer drug resistance

Targeted cancer drugs do not typically lead to long-term survival benefits owing to emergent drug resistance caused by secondary-site mutations in the targeted gene or mutations in genes downstream. René Bernards and colleagues report that suppression of *MED12* confers drug resistance to multiple targeted cancer drugs in multiple cancer cell lines (*Cell* 151, 937–950, 2012). The authors conducted an *in vitro* RNA interference (RNAi) screen targeting 8,000 genes. They found that *MED12* was the only gene whose suppression led to resistance to the ALK inhibitor crizotinib. They then performed an inverse screen with a short hairpin RNA (shRNA) library that covers all 518 human kinases to identify genes whose suppression would restore drug sensitivity. They found that suppression of *TGFBR2* resensitized $MED12^{KD}$ cells to crizotinib. Further experiments showed that treatment of multiple cancer cell lines with recombinant TGF-β conferred resistance to multiple targeted cancer drugs, as well as the widely used chemotherapy drug cisplatin. Finally, the authors tested the combination of a TGF-βR2 inhibitor with crizotinib in $MED12^{KD}$ cells and observed a synergistic inhibitory effect. The authors suggest that the combination of TGF-βR2 inhibitors with targeted tyrosine kinase inhibitors might be an effective therapy for tumors with elevated TGF-β signaling. ***PF***

Autism candidate gene resequencing

Although much effort has been made in sequencing autism exomes, it has been difficult to robustly establish ASD (autism spectrum disorder) candidate genes as bona fide genetic risk factors, as only single mutations are typically observed. Now, Evan Eichler, Jay Shendure and colleagues report ultra-low-cost ASD candidate gene resequencing of 44 genes in 2,446 ASD probands (*Science*, published online 15 November 2012; doi:10.1126/science.1227764). This resequencing method uses a modified molecular inversion probe (MIP) strategy, such that reagent costs are less than $1 per gene per sample. Applying this method to ASD probands from the Simons Simplex Collection (SSC), the authors identified 27 *de novo* mutations in 16 genes, with 59% of the mutations predicted to be truncating or disruptive of splicing. Six of the genes (*CHD8, GRIN2B, DYRK1A, PTEN, TBR1* and *TBL1XR1*) have statistically significant evidence of mutation burden; five of these are contained within the β-catenin/chromatin-remodeling network. Altogether, approximately 1% (24/2,573) of ASD probands had a mutation in 1 of these 6 genes. Because the SSC was established for families with simplex ASD and these probands typically possess higher cognitive functioning levels than other ASD cohorts, it is not known how generalizable the mutation burdens of these six genes will be in idiopathic autism. ***PF***

Written by Orli Bahcall, Pamela Feliciano, Emily Niemitz & Kyle Vogan

Summary - genes associated with Alzheimers disease

Chromosome	Gene type	Onset	% cases familial	% cases all	Product.
19	QTL	60+		40-50%	ApoE4
6	QTL	60+			TREM2
14	dominant	30-60	70-80%	5-10%	Presenilin 1 (membrane proteins)
1	dominant	40-70	20%	2-3%	Presenilin 2
21	dominant	45-65	2-3%	<1%	APP (amyloid precursor protein)

+ 9 QTLs identified by SNPs for late-onset, all with very small effect

For late onset: ~33% of risk attributable to genetic effects has been identified

Pathway analyses implicate cholesterol metabolism, innate immune response (inflammatory processes)

no mouse model that shows all AD characteristics (+12 partial models)

C elegans model worked on here , Chris Link lab

Neuropsychopharmacology (2011) 36, 2169–2186

www.neuropsychopharmacology.org

Increasing CREB Function in the CA1 Region of Dorsal Hippocampus Rescues the Spatial Memory Deficits in a Mouse Model of Alzheimer's Disease

Adelaide P Yiu[1,2], Asim J Rashid[1,2,3] and Sheena A Josselyn*[1,2,3]

[1]*Program in Neurosciences and Mental Health, Hospital for Sick Children, Toronto, Ontario, Canada;* [2]*Institute of Medical Sciences, University of Toronto, Toronto, Ontario, Canada;* [3]*Department of Physiology, University of Toronto, Toronto, Ontario, Canada*

The principal defining feature of Alzheimer's disease (AD) is memory impairment. As the transcription factor CREB (cAMP/Ca^{2+} responsive element-binding protein) is critical for memory formation across species, we investigated the role of CREB in a mouse model of AD. We found that TgCRND8 mice exhibit a profound impairment in the ability to form a spatial memory, a process that critically relies on the dorsal hippocampus. Perhaps contributing to this memory deficit, we observed additional deficits in the dorsal hippocampus of TgCRND8 mice in terms of (1) biochemistry (decreased CREB activation in the CA1 region), (2) neuronal structure (decreased spine density and dendritic complexity of CA1 pyramidal neurons), and (3) neuronal network activity (decreased *arc* mRNA levels following behavioral training). Locally and acutely increasing CREB function in the CA1 region of dorsal hippocampus of TgCRND8 mice was sufficient to restore function in each of these key domains (biochemistry, neuronal structure, network activity, and most importantly, memory formation). The rescue produced by increasing CREB was specific both anatomically and behaviorally and independent of plaque load or Aβ levels. Interestingly, humans with AD show poor spatial memory/navigation and AD brains have disrupted (1) CREB activation, and (2) spine density and dendritic complexity in hippocampal CA1 pyramidal neurons. These parallel findings not only confirm that TgCRND8 mice accurately model key aspects of human AD, but furthermore, suggest the intriguing possibility that targeting CREB may be a useful therapeutic strategy in treating humans with AD.

Neuropsychopharmacology (2011) **36,** 2169–2186; doi:10.1038/npp.2011.107; published online 6 July 2011

Keywords: memory; hippocampus; watermaze; CREB; Alzheimer's disease; dendritic spines

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in the aging population. Although the precise molecular precursors of AD remain elusive, the majority of evidence indicates that β-amyloid (Aβ) has a key role in AD pathogenesis (Price *et al*, 1998; Mucke *et al*, 2000; Tanzi and Bertram, 2001; Hardy and Selkoe, 2002; Zhang *et al*, 2011). Aβ is formed by the sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretases (Kang *et al*, 1987; Goate *et al*, 1991; Rossner *et al*, 1998; Selkoe, 1998; Mills and Reiner, 1999). Mutations in the genes encoding APP or presenilins (proteins that form part of the γ-secretase complex) increase Aβ levels (Citron *et al*, 1992, 1997; Cai *et al*, 1993; Suzuki *et al*, 1994) and are thought to cause familial forms of AD (Price *et al*, 1998; Hardy and Selkoe, 2002). Although increased levels of Aβ may eventually trigger cell death, memory impairment is often observed in AD patients before frank neurodegeneration (Cullen *et al*, 1997; Itoh *et al*, 1999; Selkoe, 2002; Vitolo *et al*, 2002). These findings suggest that high levels of Aβ negatively impact the molecular mechanisms required for normal memory formation.

CREB (cAMP/Ca^{2+} responsive element-binding protein) is a transcription factor that is critical for memory formation across a wide range of species (Dash *et al*, 1990; Bourtchuladze *et al*, 1994; Yin *et al*, 1995; Harum *et al*, 2001; Sekeres *et al*, 2010). Several distinct lines of evidence implicate mis-regulation of CREB function in AD. First, there is decreased CREB activation in post-mortem hippocampal tissue from AD patients (Yamamoto-Sasaki *et al*, 1999; Satoh *et al*, 2009). Second, analysis of an 'AD transcriptome' (produced from genome-wide expression profiling of CA1 hippocampal tissue from non-familial (sporadic) AD patients (Blalock *et al*, 2004)) identified CREB as the most highly connected hub in the AD transcriptome network (Satoh *et al*, 2009). Similar genomic network analysis shows a tight correlation between the

*Correspondence: Dr SA Josselyn, Program in Neurosciences and Mental Health, Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G 1X8, Canada, Tel: +416 813 7654, Fax: +416 813 7717, E-mail: sheena.josselyn@sickkids.ca
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Environmental risk factors for late-onset AD, other forms of dementia and cognitive decline

- overweight and/or diabetes in midlife
  increases risk of AD x1.71 (Hassing et al, 2009)

- poor nutrition
  - Swedish study: low B12 and folate levels linked to poor memory in people with risk alleles for AD.
  - 10% of older people have vit B deficiencies (low levels)

- hearing loss
  mild x2 moderate x3 severe x5 risk of dementia
  controlled for age, diabetes, hypertension

- social isolation

- cardiovascular disease, hypertension

Suggested preventative measures

- physical exercise – strongest evidence
- cognitive enrichment exercises
- social activities
- drink coffee?
- melatonin?

- these may increase chances of healthy brain – not shown to prevent AD

LETTER

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Neuronal basis of age–related working memory decline

Min Wang[1], Nao J. Gamo[1], Yang Yang[1], Lu E. Jin[1], Xiao-Jing Wang[1], Mark Laubach[1,2], James A. Mazer[1], Daeyeol Lee[1] & Amy F. T. Arnsten[1]

Many of the cognitive deficits of normal ageing (forgetfulness, distractibility, inflexibility and impaired executive functions) involve prefrontal cortex (PFC) dysfunction[1–4]. The PFC guides behaviour and thought using working memory[5], which are essential functions in the information age. Many PFC neurons hold information in working memory through excitatory networks that can maintain persistent neuronal firing in the absence of external stimulation[6]. This fragile process is highly dependent on the neurochemical environment[7]. For example, elevated cyclic-AMP signalling reduces persistent firing by opening HCN and KCNQ potassium channels[8,9]. It is not known if molecular changes associated with normal ageing alter the physiological properties of PFC neurons during working memory, as there have been no *in vivo* recordings, to our knowledge, from PFC neurons of aged monkeys. Here we characterize the first recordings of this kind, revealing a marked loss of PFC persistent firing with advancing age that can be rescued by restoring an optimal neurochemical environment. Recordings showed an age-related decline in the firing rate of DELAY neurons, whereas the firing of CUE neurons remained unchanged with age. The memory-related firing of aged DELAY neurons was partially restored to more youthful levels by inhibiting cAMP signalling, or by blocking HCN or KCNQ channels. These findings reveal the cellular basis of age-related cognitive decline in dorsolateral PFC, and demonstrate that physiological integrity can be rescued by addressing the molecular needs of PFC circuits.

Our society is rapidly ageing, with the number of seniors in the United States expected to double by 2050 (United States census, http://www.census.gov/population/www/pop-profile/elderpop.html). At the same time, the information age requires increasing organizational skills to deal with even basic needs such as medical care and paying bills. However, executive and working memory functions decline early in the normal ageing process[10–13], beginning in middle age[14,15]. Thus, cognitive changes with advancing age may be costly, forcing retirement from demanding careers and jeopardizing the ability to live independently in an increasingly complex society. Ageing monkeys provide an ideal model to reveal the neurobiology of normal ageing, as they have a highly developed PFC, but are not subject to age-related dementias[16]. Thus, one can be certain that cognitive changes are the result of normal ageing and not incipient Alzheimer's disease. Like humans, monkeys begin to develop deficits in executive function as early as middle age[17]. Both aged monkeys[18,19] and humans[3,20] are impaired on working memory tasks that require constant updating of the contents of memory (Supplementary Information), bringing to mind information from longer-term stores (for example, where did I leave my car keys this time?), or keeping in mind a recent event (for example, remembering a new phone number).

In primates, spatial working memory depends on the highly evolved dorsolateral PFC[6] (Fig. 1a). Spatial working memory performance (Fig. 1b) relies on networks of pyramidal neurons that interconnect at dendritic spines (Fig. 1c), and excite each other to keep information 'in mind', that is, generating persistent spiking activity over a delay period in a working memory task[6] (Fig. 1d). This ability to maintain information that is no longer in the environment is a fundamental process needed for abstract thought and flexible responding[4]. Intracellular signalling pathways modulate the physiological strength of these recurrent, excitatory PFC network connections[9]. Recent data show that increased cAMP signalling weakens network connectivity by opening potassium channels, whereas inhibiting cAMP signalling and/or closing these channels strengthens connectivity and cognitive ability[9] (Fig. 1e). Specifically, cAMP signalling seems to weaken persistent firing and impair working memory by increasing the open state of HCN (hyperpolarization-activated cyclic nucleotide-gated) channels that are localized on spines where networks interconnect[8]. Recent data indicate that HCN channels may also gate synaptic inputs through interactions with KCNQ channels, whose open state is increased by cAMP-activating protein kinase A (PKA)[21]. Studies indicate that cAMP signalling is disinhibited in the aged PFC[22]. Noradrenergic α_{2A} receptor inhibition of cAMP may be reduced from loss of α_{2A} receptors in the aged PFC[23], and decreased excitation of noradrenergic neurons[24].

There have been few electrophysiological recordings from aged PFC neurons owing to the demanding nature of this procedure. Recordings from rat orbital PFC found reduced flexibility in aged neurons[25]. However, there have been no *in vivo* recordings from the aged dorsolateral PFC, even though behavioural data indicate that this region is particularly vulnerable to normal ageing. *In vitro* recordings from dorsolateral PFC neurons found relatively subtle changes in excitability with advancing age[26], but their consequences for executive function must be observed in a cognitively engaged circuit. Here we perform the first physiological characterization of PFC neuronal response during a working memory task in young adult, middle-aged and aged monkeys.

Monkeys (*Macaca mulatta*, $n = 6$) were trained to perform a spatial working memory task in which they have to remember a spatial location over a brief delay period; the spatial location changes randomly on each trial (Fig. 1a). Two animals were young adults (7- and 9-year-old males), two were middle-aged (12- and 13-year-old males), and two were aged (17-year-old male, 21-year-old female). Short delays (2.5 s) were used in all age groups to ensure similar performances (>85% per cent correct) across age groups. Neurons ($n = 301$) were recorded from area 46, the dorsolateral PFC subregion most needed for visuospatial working memory (Fig. 1a). Neurons were characterized based on task-related firing as responsive during (1) the visuospatial cue period, (2) the delay period when the spatial position was being remembered, and/or (3) the motor response period. Some neurons fired only during cue presentation (CUE cells, $n = 28$), whereas most neurons fired during the delay period as well as to the cue and/or response periods (DELAY cells, $n = 273$). Persistent firing during the delay period is of particular interest, as it is required for working memory[6]. Many PFC DELAY neurons increased their activity during the memory of one spatial location (its preferred direction), but not other locations (the 'anti-preferred' direction, 180° away from the preferred direction; Fig. 1d).

[1]Department of Neurobiology, Yale University School of Medicine, New Haven, Connecticut 06510, USA. [2]The John B. Pierce Laboratory, New Haven, Connecticut 06510, USA.