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Review

The neurobiology of aging

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Abstract

Basic principles of the neurobiology of aging were reviewed within selected topic areas chosen for their potential relevance to epileptogenesis in the aging brain. The availability of National Institute on Aging-supported aged mouse and rat strains and other biological resources for studies of aging and age-associated diseases was presented, and general principles of animal use in gerontological research were discussed. Neurobiological changes during normal brain aging were compared and contrasted with neuropathological events of Alzheimer's disease (AD) and age-associated memory impairment (AAMI). Major themes addressed were the loss of synaptic connections as vulnerable neurons die and circuits deteriorate in AD, the absence of significant neuron loss but potential synaptic alteration in the same circuits in AAMI, and the effects of decreased estrogen on normal aging. The "calcium hypothesis of brain aging" was examined by a review of calcium dyshomeostasis and synaptic communication in aged hippocampus, with particular emphasis on the role of L-type voltage-gated calcium channels during normal aging. Established and potential mechanisms of hippocampal plasticity during aging were discussed, including long-term potentiation, changes in functional connectivity, and increased gap junctions, the latter possibly being related to enhanced network excitability. Lastly, application of microarray gene chip technology to aging brain studies was presented and use of the hippocampal "zipper slice" preparation to study aged neurons was described.

Keywords: Geriatric; Epilepsy; Elderly; Again brain; PK; Clinical trials

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Contents

1. Introduction

In order to provide an improved understanding of the potential neurobiological mechanisms that may cause or contribute to the development of epilepsy in the geriatric population, an overview of the neurobiology of aging was presented. This overview began with a review of principles of animal use in gerontological research and subsequently focused on important aspects of the anatomy, physiology, and molecular biology of aging brain.

2. Animal models in gerontological research

Rodent models provide potent tools for investigating the genetics, cell biology, physiology, and behavioral biology of normal aging and age-associated diseases. Rat and mouse models also have a long history of contributing to the study of epilepsy, a complex disease with multiple etiologies. There are several variables requiring careful consideration when using rodent models to study geriatric epilepsy. The first consideration is animal age. Young control animals should be fully mature, and aged animals should not be too old because distinguishing the effects of advanced age from the effects of underlying diseases can be difficult. Characterization of age-related changes should also include middle-aged animals to help establish the time frame in which these age-related changes occur. Sample sizes of animals should be large enough to allow for mortality in the aged group.

The genetic background of animals is of paramount importance in study design because it can greatly influence many physiological variables and can define what types of studies are possible. Inbred rodents are used most commonly in biomedical research because they provide genetic uniformity, which facilitates interpretation of results and allows small sample sizes to represent the population. However, the genetic uniformity of inbred mice and rats is associated with a high incidence of strain-specific pathology, and strains vary greatly from one another in both pathological and normal values. When choosing a strain for a particular study, strain-specific characteristics pertinent to experimental design and interpretation of data should be considered carefully. One concern regarding strain differences is the type and frequency of age-associated pathology. Table 1 summarizes some major strain-specific pathologies in aged rats; the example of pituitary adenomas indicates that there can be both strain and gender differences, which could influence experimental outcomes in studies of convulsive disorders.

There are differences between rodent strains in behavioral characteristics, learning, the number and proliferative potential of stem cells in the adult brain, the aging of brain function, and susceptibility to seizure disorders. The maximal electroshock seizure threshold varies greatly among strains; for example,

Table 1
Strain-specific prevalence of common pathologies

	F344[a] (%)	BN[a] (%)	F344BN F1[b] (%)
Pituitary adenoma (males)	12.9	1.2	19
Pituitary adenoma (females)	33.9	16	23
Glomerulonephropathy (males)	56	0	34
Glomerulonephropathy (females)	22	0	19
Hydronephrosis (males)	0	62	45

[a] From Lipman et al. (1999).
[b] From Lipman et al. (1996).

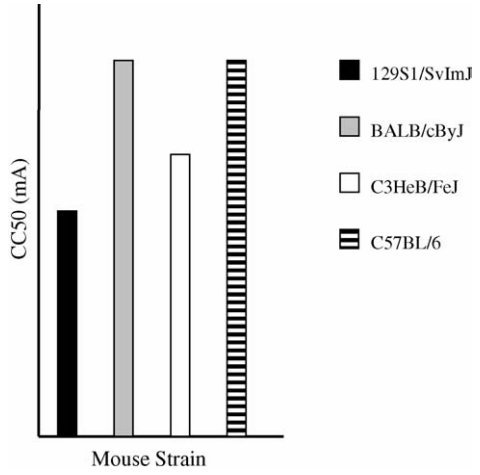

Fig. 1. CC50 for maximal hind limb extension seizure. Data derived from a study in the Mouse Phenome Database, showing the relative sensitivity to electrogenic seizures (Frankel and White, 2003).

there is a three-fold difference between DBA/2J (low) and C57BL/6J (high) mice strains (Ferraro et al., 2002). C57BL/6 and DBA/2 mice also differ greatly in susceptibility to audiogenic seizures; C57BL/6 mice are almost completely resistant to audiogenic seizures, whereas DBA/2 mice are extremely sensitive (Seyfried et al., 1986). Such strain differences have been exploited for gene discovery (Seyfried et al., 1986).

Frankel and White (2003) analyzed strain differences in sensitivity to electrogenic seizures, illustrating differences such as the amount of stimulation required for hindlimb extension seizures (Fig. 1). Of note is the difference in sensitivity between 129S1/SvImJ and C57BL/6 mice. Most knockout mice are generated by injection of 129S1/SvImJ embryonic stem cells into C57BL/6 embryos, resulting in 129S1/SvImJ × C57BL/6 chimeric mice. Even after breeding the knocked-out gene to homozygosity, the genetic background is a mixture of 129S1/SvImJ and C57BL/6. In generating a knockout to study the role of a specific gene in seizures, the mixed background would be confounding. These strain differences illustrate why it is important to cross knockout mice onto a uniform genetic background and why choice of that background can influence results.

Husbandry, diet, and environmental conditions can influence the health and behavior of rodents and affect experimental outcomes. Caloric restriction (CR) extends the life span of rodents and reduces the incidence of and/or delays the onset of many age-related pathologies (Lipman et al., 1996, 1999; Turturro et al., 1999). CR may be a particularly useful paradigm for investigating physiological influences on geriatric epilepsy; for example, raising seizure-prone El mutant mice with CR delayed the onset of seizures in response to handling (Greene et al., 2001). Ketogenic diets give a similar result, and it is likely that manipulating the energy sources in the brain alters multiple metabolic pathways with the potential to influence the genesis of seizures. The rodent model is a potent tool to investigate these pathways because the diet and environment can be controlled strictly.

Environmental enrichment, including social housing, exercise wheels, food enrichment, toys, activity stimuli, and even training, has been documented to reduce the effects of brain aging on plasticity and cognition (Van Praag et al., 2000; Kempermann et al., 2002). Three weeks of environmental enrichment resulted in an increased volume of the dentate granule cell layer and suppression of seizures after lesioning with kainate (Young et al., 1999). Such findings illustrate important considerations for the housing of rodents and suggest the potential use of cell transplantation and other approaches aimed at ameliorating seizure-induced brain damage.

The National Institute on Aging (NIA) Resources page (http://www.nia.nih.gov/research/resources.htm) provides a link to information on NIA-supported biological resources for studies on aging and age-associated diseases. The NIA aged rodent colonies provide barrier-raised aged rats and mice of a limited number of strains: BN, F344, and F344BNF1 rats, and C57BL/6, BALB/cBy, CBA, DBA/2, B6C3F1, B6D2F1, and 4-way cross mice. There is also a calorically restricted (CR) rat colony with rats raised under 40% CR from 4 months of age, as well as ad libitum-fed controls. NIA resources include: a tissue bank from the aged rodent colonies, which provides flash frozen tissue and will soon include tissue arrays; the NIA Microarray Facility providing mouse cDNA arrays; and the Aged Cell Bank, which maintains a large number of human and animal cell lines, including cell lines from patients with different types of convulsive disorders. Assistance in the use of any of these resources is available at rodents@nia.nih.gov.

3. Life and death of neurons in the aging cerebral cortex

In order to understand the neurobiological underpinnings of epilepsy in the geriatric population, it is important to review certain neuropathological events that lead to neurodegenerative disorders such as Alzheimer's disease (AD), and how these events are distinguished from the neurobiological changes associated with normal aging that may be related to geriatric epilepsy or functional decline, such as age-associated memory impairment (AAMI). Data supporting three major conclusions regarding brain aging are discussed: (1) in AD, synaptic connections are lost as selectively vulnerable neurons die and circuits deteriorate; (2) AAMI occurs in the absence of significant neuron loss, yet may involve synaptic alteration in the same circuits; and (3) endocrine senescence (e.g., decrease in circulating estrogen) interacts with neural aging and may impact both cognition and related neural circuits.

AD is marked by significant death of neurons in certain cortical regions and layers in which a particularly vulnerable neuronal phenotype is prevalent. The circuits that are most vulnerable to degeneration are the perforant path, which connects the entorhinal cortex with the hippocampus, and the long corticocortical projections that link association cortices such as inferior temporal cortex and prefrontal cortex (Morrison and Hof, 2002). As these circuits degenerate, there is initially a memory defect from the perforant path degeneration, followed by dramatic loss of cognitive abilities as the corticocortical circuits degenerate. Patients with a moderate memory defect but lacking dementia are often classified as having mild cognitive impairment. These patients have received a great deal of attention because they are the key to understanding the difference between AD and AAMI. Mild cognitive impairment can represent the fairly stable condition of AAMI or the early stage of progressive AD, which will become much more severe over time. As interventions emerge that are appropriate for AD, as opposed to AAMI, this clinical distinction within the mild cognitive impairment group will become of paramount importance.

AAMI is seen in humans, monkeys, and rodents and is not accompanied by significant neuron loss, but is more likely caused by synaptic changes. The corticocortical circuits that are vulnerable in AD are also affected in normal aging in that spines on the neurons providing these circuits are lost, but the circuits are still largely intact (Fig. 2, Duan et al., 2003). In addition, there are age-related changes in the perforant path projection such as a loss of presynaptic markers in rodents, and a loss of postsynaptic glutamate receptors, particularly *N*-methyl-D-aspartate (NMDA) receptors, in monkeys (Morrison and Hof, 2002). The electrophysiological properties of hippocampal neurons reflective of NMDA receptor function are also compromised in aged animals (see Section 5 below, aging brain and plasticity).

Several studies have demonstrated the links between estrogen and the aging brain. These studies reinforce the point that many of these circuits affected by aging are amenable to restoration of function, and estrogen appears to have profound effects on these circuits. Earlier studies showed that estrogen causes an increase in spines in CA1 in young rodents and that this effect is NMDA receptor-dependent (McEwen, 2002). Our more recent studies show that aged rats do not display an increase in spine number in response to estrogen, but that CA1 synapses have more NMDA receptors after estrogen treatment in aged animals (Adams and Morrison, 2003). The inability of aged rats to form more spines in response to estrogen may be due to the fact that there are far fewer estrogen receptors in the spines of aged rats than in young rats (Adams and Morrison, 2003). Recent monkey studies have shown that aged animals are quite responsive to estrogen replacement by cognitive testing. In aged ovariectomized monkeys, estrogen restored performance in both hippocampal- and prefrontal cortex-dependent tasks compared with that demonstrated by a young intact animal (Fig. 3, Rapp et al., 2003). Analyses of spines in primates show that, unlike in the rat, hippocampal spine number increases in response to estrogen in both young and aged animals, suggesting that aged monkeys may be more responsive to estrogen than aged rats (Hao et al., 2003). In addition, estrogen leads to a dramatic increase in spine number in the prefrontal cortex, which provides a neurobiological basis for the enhanced prefrontal function seen in this model (Tang et al., 2003).

General conclusions are that: (1) both rats and monkeys exhibit AAMI and thus model similar non-AD age-associated impairments in humans (monkeys are particularly useful to model compromised prefrontal cortical function); (2) these functional losses are not

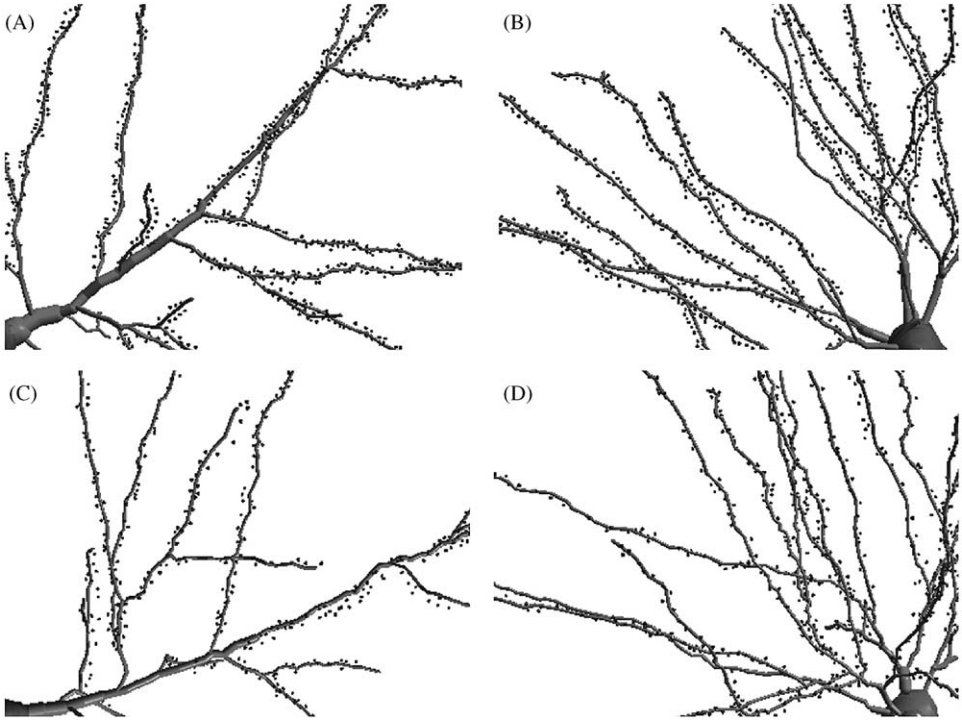

Fig. 2. Examples of apical and basal dendritic segments with all spines shown, from pyramidal neurons in macaque monkey temporal association cortex (superior temporal sulcus) that project to dorsolateral prefrontal cortex. These corticocortical circuits are vulnerable to age-related loss of spines. (A) and (B) show apical and basal dendrites, respectively, of such a neuron from a young animal. (C) and (D) show apical and basal dendrites, respectively, from a similar neuron in an aged animal. Note the comparative loss of spines in the aged dendrites (C) and (D). Quantitative analyses showed that these corticocortically projecting neurons in aged animals consistently had 30–40% fewer spines in both the apical and basal dendritic trees [Duan, H., Wearne, S.L., Rocher, A.B., Macedo, A., Morrison, J.H., Hof, P.R., Age-related Dendritic and Spine Changes in Corticocortically Projecting Neurons in Macaque Monkeys, *Cerebral Cortex*, 2003, Vol. 13, No. 9, pp. 950–961, by permission of Oxford University Press].

due to frank neuron loss or circuit degeneration but more likely due to molecular and structural alterations of select hippocampal and neocortical synapses; (3) NMDA receptor-mediated neurotransmission is particularly vulnerable to aging; and (4) the primate data on estrogen replacement suggest that age-related cognitive decline and the underlying synaptic alterations are amenable to intervention and restoration of cognitive performance.

4. Calcium dyshomeostasis and synaptic communication in aged hippocampus

Research on Ca^{2+} dysregulation in CA1 pyramidal neurons of aged rodents has contributed to the formulation of the "Ca^{2+} hypothesis of brain aging and dementia" (Landfield and Pitler, 1984; Thibault and Landfield, 1996). Proposed initially in the early to mid 1980s and based on the results of a handful of investigators (Khachaturian, 1984; Landfield and Pitler, 1984; Michaelis et al., 1984; Gibson et al., 1986), the hypothesis states that alterations in Ca^{2+}-dependent processes during aging may affect Ca^{2+} signaling pathways and, consequently, impair plasticity (Landfield, 1987; Disterhoft et al., 1993; Thibault et al., 1998). In turn, impaired neuronal communication could have negative impacts on cognition and memory.

Over the last 20 years, numerous aspects of intracellular Ca^{2+} regulation and signaling have been studied and elucidated during brain aging. Mecha-

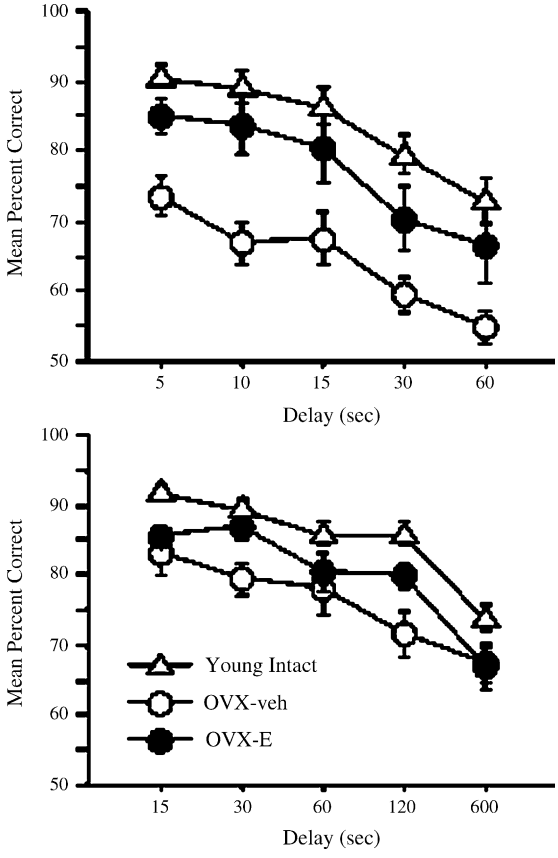

Fig. 3. Neuropsychological results in aged ovariectomized female monkeys with vehicle and aged ovariectomized monkeys with estrogen replacement, compared to young intact animals. The top panel shows results from delayed response (DR), a task dependent on prefrontal cortex, and the bottom panel shows data from delayed non-matching to sample (DNMS), a hippocampus-dependent task. The DR data show a substantive increase in performance with estrogen treatment, effectively restoring performance to that of young animals. The DNMS data show that the estrogen-treated animals performed significantly better than the non-treated animals at delays of 30 and 120 s, again restoring performance to that of a young animal. Thus, estrogen improves cognitive deficits in aged monkeys that are linked to both hippocampus and prefrontal cortex [Reprinted from *The Journal of Neuroscience*, Vol. 23, Rapp, P.R., Morrison, J.H., Roberts, J.A., Cyclic estrogen replacement improves cognitive function in aged ovariectomized rhesus monkeys, pp. 5708–5714, copyright 2003 by the Society for Neuroscience].

nisms implicated in brain aging and/or associated with altered synaptic function and cognitive decline include Ca^{2+} uptake and release via the endoplasmic reticulum and mitochondria, plasma-membrane-associated influx and removal of intracellular Ca^{2+}, and Ca^{2+} buffering content and power. Teasing apart these different mechanisms and their relationships, and analyzing them in the context of a single cell within a defined set of Ca^{2+} microdomains and signaling pathways, has begun only recently. Moreover, it has not been clear whether these homeostatic Ca^{2+} mechanisms and their interactions influence net resting Ca^{2+} levels in intact hippocampal cells of aged animals, leaving unanswered the important question of whether Ca^{2+} concentration during aging is directly correlated with altered synaptic function.

Our studies have focused on measures of Ca^{2+} influx pathways and the consequences of Ca^{2+} dyshomeostasis on synaptic function in aged neurons. The first indirect evidence for elevated neuronal Ca^{2+} influx during aging came from measures of the slow Ca^{2+}-dependent afterhyperpolarization (AHP), which follows action potentials in CA1 pyramidal neurons and affects synaptic throughput and excitability in the hippocampus. Amplitude and duration measures of the AHP were found to be significantly larger in aged rats (Landfield and Pitler, 1984), suggesting an age-related increase in neuronal [Ca^{2+}]. In addition to the AHP, Ca^{2+}-dependent synaptic plasticity processes, such as frequency facilitation (FF), measuring the growth of the excitatory postsynaptic potential (EPSP) during 5–10 Hz trains of synaptically activated action potentials, were markedly impaired in aged animals (Landfield et al., 1986). Measurement of Ca^{2+} potentials and currents came later and provided the first direct evidence for elevated L-type Ca^{2+} currents with aging (Pitler and Landfield, 1990; Campbell et al., 1996). However, the molecular mechanisms for these elevated electrophysiological indices of Ca^{2+} signaling could not be determined without single channel analyses of the biophysical properties of voltage-gated Ca^{2+} channels (VGCCs).

Using a mild dissociation procedure for optimizing single-channel patch clamp recordings in the hippocampal slice (Gray et al., 1990), a large increase in the functional density of available L-type VGCCs in the membranes of aged neurons, relative to young-adult and mid-aged neurons, was detected (Thibault and Landfield, 1996). This increase in L-type Ca^{2+} channel activity appears to be a primary mechanism for triggering Ca^{2+} dyshomeostasis in brain aging. L-type VGCC density also correlated negatively with measures of learning, suggesting that L-type VGCC func-

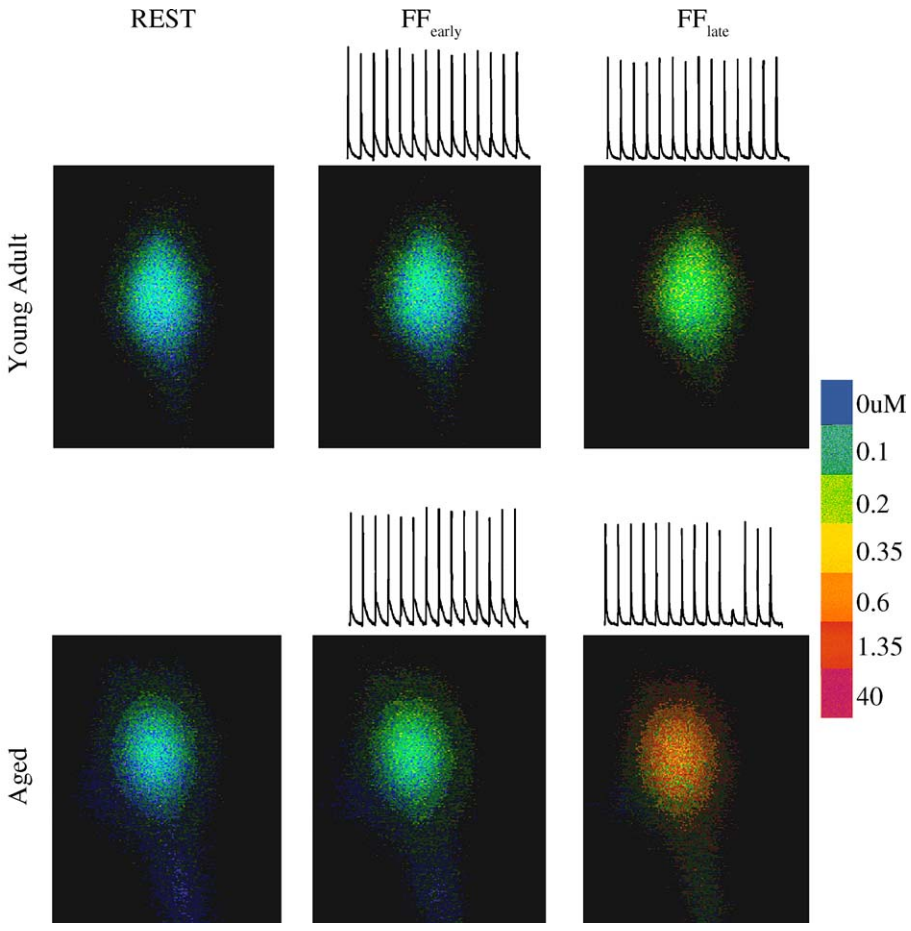

Fig. 4. Confocal Ca^{2+} imaging in intact hippocampal CA1 neurons revealed elevated patterns of Ca^{2+} during physiological activation. Cells were synaptically stimulated for 20 s at 7 Hz above action potential threshold (electrophysiology traces), which induced short-term FF of EPSPs. Rest, FF_{early} (first second of stimulation), and FF_{late} (last second of the 20 s train) images are displayed for a young adult (top row) and an aged cell (bottom row) [Reprinted from *The Journal of Neuroscience*, Vol. 21, Thibault, O., Hadley, R., Landfield, P.W., Elevated postsynaptic $[Ca^{2+}]i$ and L-type calcium channel activity in aged hippocampal neurons: relationship to impaired synaptic plasticity, pp. 9744–9756, copyright 2001 by the Society for Neuroscience].

tion contributes to age-dependent cognitive decline. However, increases in L-type Ca^{2+} channel density may not necessarily result in net elevations of intracellular Ca^{2+} levels because CA1 pyramidal neurons have multiple mechanisms for Ca^{2+} buffering and sequestration that are potentially capable of offsetting elevations in L-type Ca^{2+} channels. Thus, direct measurements of intracellular Ca^{2+} concentrations became necessary to determine the potential impact of elevated L-type VGCCs on Ca^{2+} levels and synaptic function. Using confocal laser scanning microscopy of non-dissociated slices of aged hippocampus, it was found that neuronal $[Ca^{2+}]$ transients were elevated, whereas short-term synaptic plasticity (FF) was impaired during physiological patterns of synaptic activation (see Fig. 4) (Thibault et al., 2001). This Ca^{2+}-dependent impairment in synaptic throughput could be mimicked in young-adult neurons by selectively increasing L-type VGCC activity. Thus, increased L-type VGCC channel activity appeared to act either as a primary source or trigger of elevated $[Ca^{2+}]$ transients with aging. One postsynaptic mechanism by which elevations in Ca^{2+} levels could depress EPSP amplitudes in aged neurons is the activation of a dendritic shunt carried by K^+ efflux

because Ca^{2+}-activated K^+ currents and potentials are elevated in aged CA1 neurons.

Because the "aging" phenotype could be mimicked in young-adult neurons by enhancing L-type VGCC activity, an important test of the Ca^{2+} hypothesis is the creation of a "young" phenotype in aged neurons by specifically reducing L-type Ca^{2+} channel function. This has been accomplished in some models with Ca^{2+} channel blockers (Disterhoft et al., 1993; Norris et al., 1998; Thibault et al., 1998); however, recent studies have found that the active metabolite of vitamin D (Vit D) has strong neuroprotective effects against excitotoxic challenges in hippocampal cultures, possibly mediated by reducing L-type VGCC activity (Brewer et al., 2001). Preliminary studies in vivo revealed that treatment of aged animals with Vit D reduced L-type VGCC density to levels seen in young animals, whereas Vit D had little effect in younger animals. Thus, the effect of Vit D treatment was selective for aged animals. Additionally, a decrease in AHP amplitude and an improvement in synaptic plasticity (FF) were also found in slices from aged animals treated with Vit D.

Taken together, these data suggest that Ca^{2+}-dependent processes affect Ca^{2+} signaling pathways and impair synaptic function in an aging-dependent manner, consistent with the Ca^{2+} hypothesis of brain aging and dementia. Although synaptic plasticity may be depressed in aging, elevated Ca^{2+} signals in aged neurons could trigger greater K^+ efflux, which may contribute to elevations in synchronized burst firing within the hippocampus (Patrylo et al., 1994), possibly participating in the ontogenesis of epilepsy. The role of aging-related changes in brain Ca^{2+} homeostasis in epilepsy and overall excitability remains to be elucidated.

5. Aging brain and plasticity

There are a number of neurobiological alterations responsible for memory changes that occur during aging. One function of the mammalian hippocampus is acquiring spatial knowledge, which allows us to navigate through large-scale space. Acquisition of new spatial maps is defective in aged organisms, including humans, monkeys, dogs, rats, and mice. Spatial learning and memory processing can be tested under similar conditions across a wide range of species (Barnes, 1990 and see Rosenzweig and Barnes, 2003 for reviews). A circular platform task was developed in the 1970s, specifically for aged rats, where rats can use visual cues in the room to navigate and escape into a dark tunnel. Aged rats do not remember the location of the escape tunnel, as do young rats, indicating age-related changes in learning and memory and suggesting hippocampal involvement.

Contrary to the idea that global deterioration of the brain occurs during normal aging, experimental observations indicate that there is widespread preservation of function in the aged brain and, in general, the changes that do occur are relatively specific to different subregions of the hippocampus (see Barnes, 1994; Rosenzweig and Barnes, 2003 for reviews). Additionally, compensatory processes can occur in the aged brain. The numbers of principal cells in the hippocampus do not change across the lifespan during normal aging, regardless of the species. Most physical properties of hippocampal neurons do not change with age, including resting membrane potential, input resistance, height of the action potential, time constant, and EPSP rise time and half-width. One exception to this is the increased afterhyperpolarizing potential in aged CA1 pyramidal neurons described in Section 4. Aging-related changes that do occur are primarily in connectivity and functional responsiveness. There appears to be fewer axon collaterals from entorhinal cortex synapsing on the dendrites of dentate granule cells (Fig. 5). However, with axon collateral pruning, the synaptic contacts that remain are more powerful for a given input, i.e., the amplitude of the unitary EPSP in aged animals is actually greater. Thus, in aged granule cells, there appears to be a compensatory mechanism to keep the overall throughput in the granule cells constant by the strengthening of synaptic connections. There is no similar change in the Schaffer collaterals going from CA3 to CA1, but there appears to be either fewer actual or functional synaptic contacts on CA1 pyramidal cells. The efficacy of individual synaptic contacts in CA1 does not change. Thus, CA1 synapses do not compensate for the reduction in functional synaptic connectivity in the same manner, as do granule cells.

Another area for neurobiological correlates of age-related impaired memory is the plasticity characteristics of the aged hippocampus. Much is known about the physiology, pharmacology, and molecular biol-

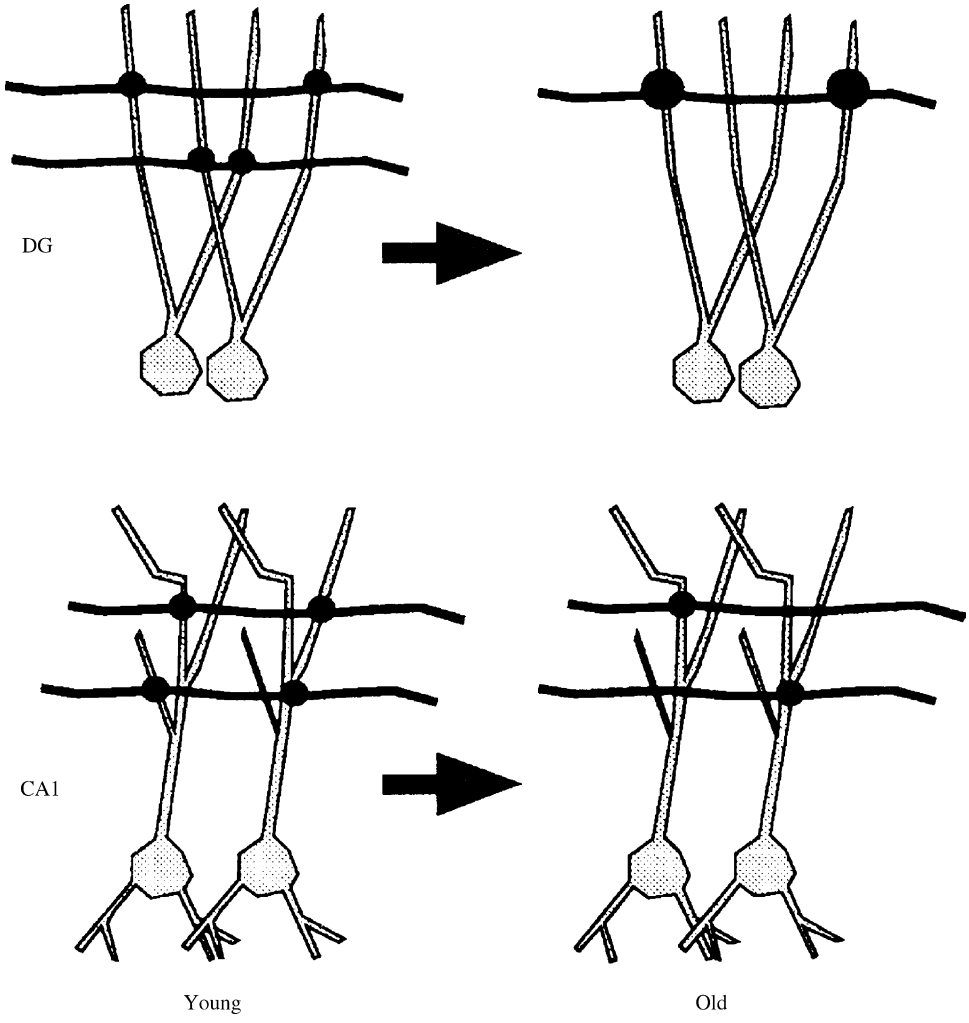

Fig. 5. Summary sketch of the differences in functional connectivity between young and old rats that have been discovered in electrophysiological experiments. Top: schematic diagram of granule cells in the hippocampus of young (left) and old (right) rats. Axonal collaterals from the entorhinal cortex are pruned in old rats, but the synaptic connections that remain are individually more powerful in the old animals (larger circles), suggesting a possible compensatory mechanism that may keep the overall throughput in these cells approximately constant during aging. Bottom: schematic diagram of CA1 pyramidal cells in the hippocampus of young (left) and old (right) rats. This is not an age-related change in the number of axonal collaterals (Schaffer collaterals) that project from CA3 pyramidal cells during aging, nor is there a difference in the synaptic strength of Schaffer collateral synapses between young and old rats. There is, however, a reduction of actual or functional synaptic contacts onto any given CA1 cell in older animals [Reprinted from *Trends in Neuroscience*, Vol. 17, Barnes, C.A., Normal aging: regionally specific changes in hippocampal synaptic transmission, pp. 13–18, 1994, with permission from Elsevier].

ogy underlying the processes of long-term potentiation (LTP) in the hippocampus. LTP at hippocampal synapses may represent the experimental activation of processes that normally subserve information storage. With respect to aged neurons, there is no deficit in inducing LTP when robust induction parameters are used; however, synaptic strength decays more quickly in aged neurons. After training young and aged animals to the same level of performance for the circular platform task, forgetting rates in escape time were about twice as fast in aged animals (Fig. 6). Within each age group, spatial memory accuracy was correlated with the decay speed of LTP in individual animals. In studies using perithreshold induction parameters, an LTP

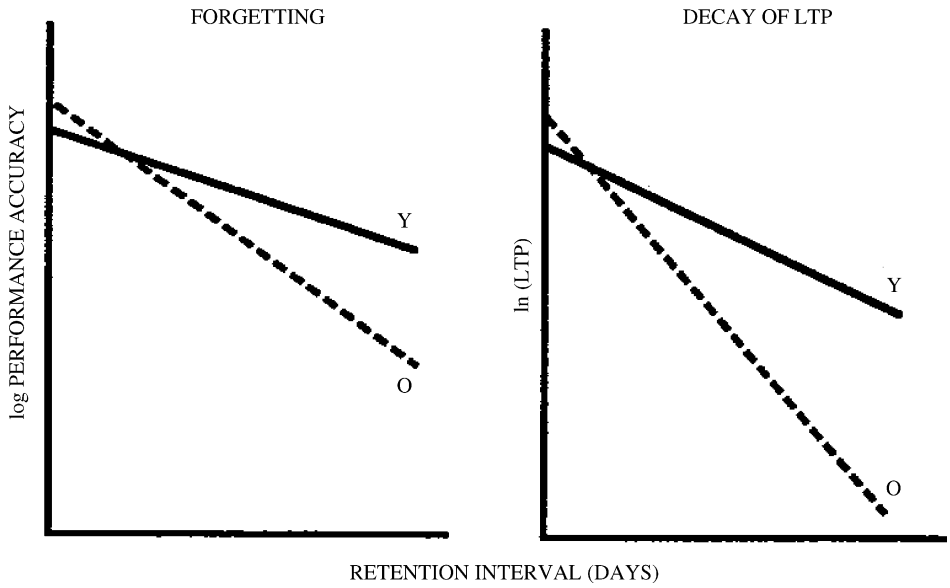

Fig. 6. Schematic drawing of spatial forgetting rates and LTP decay rates in young and old rats. Data are drawn after the data presented in Barnes and McNaughton (1985) in which young and old rats were assessed with respect to behavioral forgetting in the circular platform task, and with respect to decay of LTP at the perforant path—granule cell synapse. Old rats forget spatial information faster (over a 60-day period) and show faster decay of LTP (over a 30-day period) than do younger rats.

induction deficit was observed in aged animals. Conversely, long-term depression in aged animals is easier to induce compared to young animals. In summary, LTP is more difficult to achieve in older animals and decays more quickly when it is induced robustly (see Barnes, 2003 for review). In this way, one of the most important mechanisms for information storage in the nervous system is compromised in older animals.

Early immediate genes have been studied as potential mediators of long-lasting plasticity. *Zif* 268, a transcription factor, is robustly induced in the dorsal hippocampus after LTP-inducing stimulation. *Zif* 268 is activated powerfully in both young and aged animals and is therefore not likely to contribute to aging-related changes associated with LTP induction. However, induction of c-*fos* mRNA following LTP induction was greater in aged animals compared to young animals and was confined to the dorsal hippocampus (Lanahan et al., 1997). This selective change in the aged hippocampus suggests another signaling pathway potentially involved in LTP maintenance deficits.

Two examples of changes that occur uniformly, rather than specifically, throughout the hippocampus during aging are a decline in functional cholinergic transmission, as measured by slow EPSP amplitudes (Shen and Barnes, 1996), and a change in gap junctional connectivity, as measured by dye-filling of neurons (Barnes et al., 1987). Although the several different aging-related changes described to this point would result in decreased excitability of aged neurons, electrical connections via gap junctions may be more extensive in aged hippocampal neurons and result in an overall lowering of the threshold for action potential discharge, i.e., a mechanism demonstrating the potential for increased excitability in aged neurons. The possible relationship of increased aging-related gap junctional connectivity and enhanced network excitability with the increased expression of epilepsy in the elderly awaits further investigation.

6. Aging brain and microarray gene chips

DNA microarrays are rectangular surfaces made out of glass or plastic, and coated with chemicals (probes) that enable microarrays to detect and quantify the presence of thousands of different mRNA species. Microarrays can be divided into two groups based on their

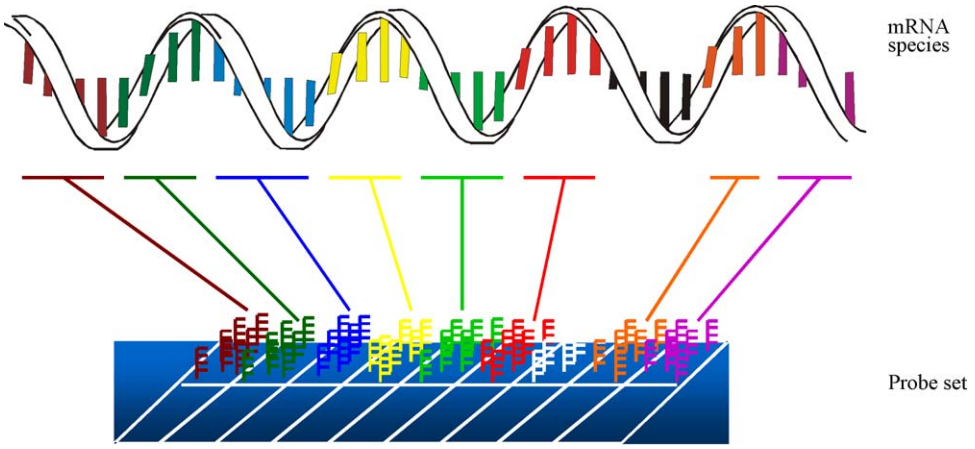

Fig. 7. Affymetrix probe set, a spot does not equal a gene. In this design, a family, or set, of probes is used to detect the presence and quantity of a particular mRNA species. Specific features (individual squares) on the probe set (lower) contain probes designed to detect discrete regions of an mRNA species (upper).

printing technologies: spotted arrays and photolithographic arrays (Affymetrix proprietary). Spotted arrays usually require two different biological samples per chip, and a ratio of their binding is calculated for each spot, whereas Affymetrix arrays measure a single sample per chip using a more intricate 'family' or 'set' of probes (Fig. 7) (Blalock, 2003). Using these approaches, researchers are able to measure a much larger portion of the transcriptome (the total mRNA that a single organism/tissue/cell type is capable of producing) simultaneously than was possible prior to microarray technology. Although microarrays represent a tremendous gain with regard to assessing the behavior of the transcriptome in various experimental and clinical settings, their use also is hindered by statistical noise and difficulties associated with bioinformatic analyses (Miller et al., 2001; Blalock et al., 2003, 2004).

In order to generate an integrative model of age-related memory problems, Affymetrix microarrays (RG-U34A) were used to examine age-related changes in hippocampal transcriptional profiles as they related to cognitive deficits in rodents (Blalock et al., 2003). Earlier studies of brain aging have confirmed the presence of markers previously implicated in aging (e.g., inflammation). However, these studies were often hindered by low replication and fold-change criteria for the selection of interesting genes, affording them little power to discover more subtle changes in the transcriptome of the aging brain. For microarray experiments, a novel statistical design in which both group testing (1-way analysis of variance [ANOVA]) and behavioral correlation (Pearson's test) strategies were used to identify Aging and Cognition Related Genes (ACRGs) was employed. Fischer 344 rats of three different ages, 4, 14, and 24 months (9–10 animals/group), were behaviorally characterized (Spatial Water Maze and the Object Memory Task). In both tasks, there was a non-significant trend towards decreased performance by mid-age rats, and a significant drop-off in cognitive performance by old age rats. Hippocampi were removed and the CA1 region isolated for microarray analysis. Each animal's tissue was hybridized to a single microarray, maintaining a 1:1 relationship between the behavior and the microarray for each animal. Although other pooling strategies could be used effectively with statistical discovery (Peng et al., 2003), maintaining this relationship between behavior and microarray data was vital for the correlation approach employed. The microarray data were filtered prior to statistical testing based on *a priori* criteria. This process allowed a reduction of the total number of statistical tests (as well as the number of false positives that arise by the error of multiple testing). Probe sets were removed that were rated absent, were undefined (expressed sequence tags [ESTs]), or did not maintain a sustained change from the young to the aged groups. These settings reduced the total number of probe sets

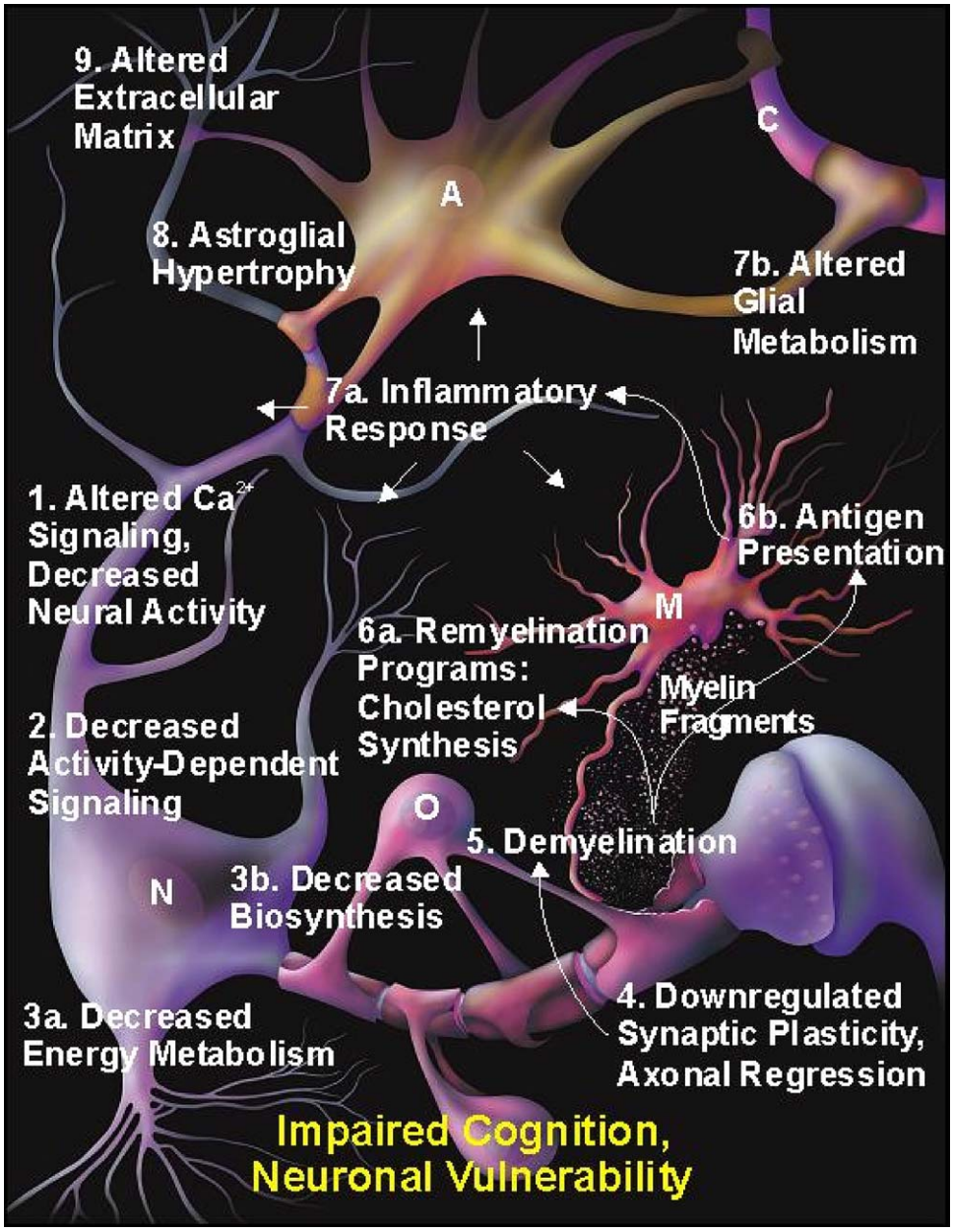

Fig. 8. Possible ontogeny of age-related cognitive decline. (1) Altered Ca^{2+} signaling in neurons (N) leads to decreased (2) activity-dependent signaling, (3a) energy metabolism, and (3b) biosynthesis. This may result in downregulated (4) synaptic plasticity and axonal regression. Our work points to a reflexive increase in (5) demyelination, stimulating both (6a) remyelination in oligodendrocytes (O) as well as (6b) antigen presentation in microglia (M) and a feed forward cycle of increasing (7a) immune response. Downstream altered glial metabolism, astrocytic hypertrophy (A) and extracellular matrix could contribute importantly to impaired cognition and neuronal vulnerability. (Reprinted from Blalock et al. (2003) with permission from J. Neurosci.)

to be tested statistically from ∼9000 to ∼2000. Statistical testing (1-way ANOVA; $p < .025$) identified 233 age-related genes, 161 of which were also correlated with behavior. This latter set was regarded as ACRGs. ACRGs were functionally grouped to determine which physiological processes were associated with the age-related decline in cognitive performance. Several categories previously shown to change with aging (e.g., ↑ inflammatory markers, ↓ energy metabolism, and ↓ neurite/ synaptic plasticity) were identified, provid-

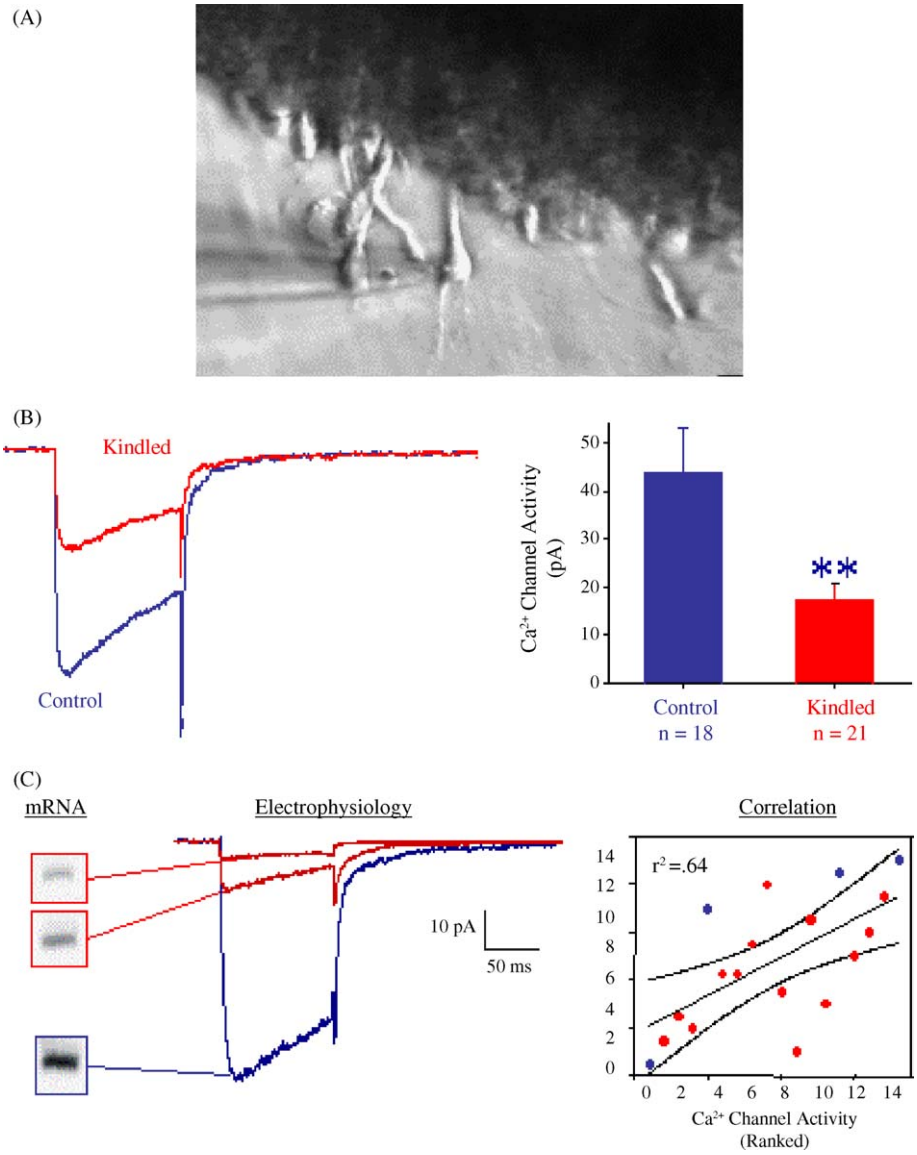

Fig. 9. Applying zipper technology to epilepsy research. (A) Photomicrograph of an 'unzipped' hippocampal slice from a kindled rat. (B) There is a highly significant (**$p < .01$; *t*-test) reduction in L-type Ca^{2+} current measured from the soma of pyramidal cells of the CA1 layer of the hippocampus using cell-attached patch clamp recording technology. (C) Subsequent cell collection and quantification of L-type mRNA (α_{1D}) reveals a significant correlation between mRNA and average ensemble current amplitude from control (blue) and kindled (red) animals ($p < 0.01$; Spearman correlation).

ing a positive control for other, novel categories that were also identified (e.g., ↓ transcriptional regulators and ↑ cholesterol synthesis). Interestingly, for nearly every gene identified as an ACRG, at least half of the total change in expression had occurred by mid-age, a time point at which no significant cognitive deficits had been observed. Thus, changes in gene expression at the transcriptional level may precede and predict later cognitive decline. Together, these findings lead to the proposal of an integrated model of age-related cognitive decline (Fig. 8) (Blalock et al., 2003).

Because of the lack of neuronal specificity in tissue collection associated with microarrays, a parallel electrophysiological and molecular characterization of single neurons obtained from kindled rats using 'zipper slice' technology was developed (Chen et al., 2000; Blalock et al., 2001). In the microarray studies, a brain region (hippocampal CA1) relatively enriched in neurons compared with other central nervous system (CNS) cell types (e.g., oligodendrocytes and microglia) was excised; however, other cell types are present in the homogenized extract and may dilute or occlude neuron-specific changes (Chen et al., 2000; Blalock et al., 2001). Based on 'zipper slice technology' (Gray et al., 1990), a method for recording from and collecting intact single neurons from adult, aged, and kindled rat hippocampus was developed (Chen et al., 2000; Blalock et al., 2001). Experiments examining the role of L-type Ca^{2+} channels in a kindling model of epilepsy clearly demonstrated a significant decrease in this current in long-term kindled animals (1.5–3 months), as well as a significant correlation between L-type current amplitude and quantity of α_{1D} mRNA measured in each cell (Fig. 9).

Thus, the noise inherent in microarray data experiments can be countered by sufficient biological replication and statistical analysis. Using these approaches, a new, integrative model of brain aging was developed in which axonal regression may promote a demyelinating response (Fig. 8) that is the key trigger for inflammatory changes seen in the aging brain (Chen et al., 2000; Blalock et al., 2001). Further, these data suggest that critical transcriptional events occur at or before mid-age and predict later cognitive decline. In addition, the zipper slice preparation seems ideally suited to the study of both physiological and transcriptional changes in neurons. Future studies of electrophysiologically characterized neurons will include strategies for mRNA amplification and assessment by microarray technology.

7. Conclusion

This very brief summary of the neurobiology of aging demonstrates that many new and exciting tools are becoming available to elucidate the mechanisms of brain aging. Hopefully this will inspire a greater use of the appropriate models to better understand the changes occurring in the brain with the passage of time and, moreover, the mechanisms of epileptogenesis in the aging brain.

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