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Research report

A novel mouse model of the aged brain: Over-expression of the L-type voltage-gated calcium channel $Ca_V1.3$

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HIGHLIGHTS

- Generation of a novel transgenic mouse model of the "aged brain".
- Expression of HA-tagged $Ca_V1.3$ driven by αCamKII promoter.
- Results in ~50% increase in $Ca_V1.3$ channels in forebrain tissue.
- Mice exhibit normal behavioral responses in a series of neurobattery tasks.

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ABSTRACT

The aged population is growing rapidly, which has sparked tremendous interest in elucidating mechanisms of aging in both the body and the brain. Animal models have become an indispensable tool in biomedical science, but because of the cost and extended timeframe associated with aging animals to appropriate time points, studies that rely on using aged animals are often not feasible. Somewhat surprisingly, there are relatively few animal models that have been specifically engineered to mimic physiological changes known to occur during "normal" aging. Developing transgenic animal models that faithfully mimic key aspects of aging would likely be of great utility in studying both age-related deficits in the absence of overt pathology as well as an adjunct for transgenic models of diseases where aging is a primary risk factor.

In particular, there are several alterations in the aged brain that are amenable to being modeled genetically. We have focused on one key aspect that has been repeatedly demonstrated in aged animals – an increase in the L-type voltage-gated calcium channel $Ca_V1.3$. Here we present a novel transgenic mouse line in which expression of $Ca_V1.3$ is increased by approximately 50% in the forebrain of young mice. These mice do not display any overt physical or non-cognitive deficits, exhibiting normal exploratory behavior, motor function, and affective-like responses, suggesting that these mice can be successfully deployed to assess the impact of an "aged brain" in a variety of conditions.

1. Introduction

As people age, they often experience declines in physical, physiological, and mental function which not only reduce the lifespan but also adversely impact the quality of life. The aged population is one of the fastest growing segments of society, which has been predicted to double in size by the year 2050, potentially representing over 20% of the total population in the United States [1]. As such, there has been increased interest in elucidating mechanisms that underlie age-related alterations so that potential therapeutic inter-

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ventions can be identified and developed to ameliorate declining function.

Animal models have become an invaluable tool in biomedical science, and are used to study myriad aspects of biological mechanisms. Often, animal models are generated by introducing mutations into the genome to alter the function of target proteins or processes or to replicate mutations linked to specific human disease states. The "aged" phenotype, however, comprises a constellation of various changes throughout the body and brain that are complex and often interdependent, making it difficult to produce an accurate model. Thus, researchers interested in studying aging often have to literally "age" animals, simply waiting for them to reach a time point that is comparable to that of interest in humans. In the case of rodents, which are commonly used as animal models, this time point is generally 2–3 years, making this a very time-consuming and expensive experimental design.

One approach that has been taken in order to accelerate the time frame necessary for generating phenotypically aged mice is selective breeding. The senescence-accelerated-prone (SAMP) mouse lines were established by continuous interbreeding of littermates, which were selected on the basis of early senescence, shortened lifespan, and the development of age-associated pathologies, for many successive generations [2]. This process yielded a number of separate lines that each mimic specific characteristics of aged animals, including both physical deficits and cognitive deficits [3]. However, it is important to note that while these mice mimic some aspects of aging, it is difficult to determine whether the same mechanisms that underlie aging are contributing to the deficits observed in the SAMP mice. Thus, alterations in the expression, activity, and/or function of genes and proteins in the SAMP lines need to be validated by comparison to control mouse lines. While this represents a powerful unbiased method for identifying potential targets of interest, the selection of the "control" mouse line(s) introduces another significant caveat. Because the SAMP lines are a result of selective breeding, all mice in these lines are senescence-accelerated; therefore, there are no unaffected littermates to use as controls. Instead, control mice must be selected from other (genetically inbred) lines, each of which may have distinct mechanisms that contribute to an "aged" phenotype resulting in different catalogs of potential targets depending on the "control" chosen. A final crucial point is that each of the SAMP lines generally comprises multiple alterations that may contribute to the observed phenotypes. While this is similar to aging in which multiple factors likely interact to produce observed deficits, it confounds the understanding of the mechanism(s) responsible for specific age-related impairments. For example, the SAMP8 mouse line has garnered considerable interest because these mice exhibit cognitive deficits (particularly in learning and memory), which is a hallmark of aging in humans [4,5]. However, these mice display both an increase in oxidative stress as well as an increase in amyloid beta [5], making it is difficult to determine which mechanism is responsible for the deficits in learning and memory, and, moreover, to precisely elucidate the relative contribution of these processes to the cognitive decline that is observed in aged subjects.

In light of these limitations, our goal was to design a mouse model in which functional as well as mechanistic aspects of brain aging could be interrogated. There are many well-documented age-related changes that occur in the brain [6–8] and a number of these alterations could be modeled transgenically. We have chosen to focus our initial efforts on one key aspect of the aged brain: dysregulation of neuronal calcium (Ca^{2+}) homeostasis [9–11]. As a ubiquitous signaling molecule [12,13], a crucial regulator of gene transcription [14,15], and a critical modulator of both neuronal excitability [16] and plasticity [17], even small changes in Ca^{2+} homeostasis can significantly alter brain function. Several lines of evidence support the idea that Ca^{2+} homeostasis is dysregulated in the aged brain and that this contributes to age-related impairments in brain function (for example, the deficits in learning and memory that are often observed during aging), even in the absence of overt pathology. Calcium imaging experiments in the hippocampus showed that neurons from aged rats exhibited a significantly larger increase in intracellular Ca^{2+} concentration in response to depolarization than neurons from young rats [17]. This difference was only observed when the neurons fired action potentials, which suggested that the high voltage-activated class of voltage-gated calcium channels was involved [17]. Indeed, additional electrophysiological experiments demonstrated that the increase in whole-cell calcium currents was a result of an increase in the density of L-type voltage-gated calcium channels (L-VGCC) [18]. Further, the magnitude of the increase in calcium current and channel density was correlated with the degree of cognitive impairment in a hippocampus-dependent learning and memory task (the Morris water maze) [18]. Another line of evidence comes from electrophysiological recordings of the slow afterhyperpolarization (sAHP), a key determinant of neuronal excitability [19], which has been shown to require activation of L-VGCCs [20–23]. The sAHP in neurons from aged animals is significantly increased relative to that in young animals [24–27], consistent with the model of an increase in the number of available L-VGCCs that in turn activate more of the current underlying the sAHP. Interestingly, the magnitude of the sAHP recorded from hippocampal neurons has also been shown to be correlated with the degree of impairment in hippocampus-dependent learning and memory tasks (trace eyeblink conditioning and trace fear conditioning) – animals with a larger sAHP are impaired relative to those with a smaller sAHP [28,29].

There are two primary L-VGCC pore forming subunits that are expressed in the mammalian brain: $Ca_V1.2$, which comprises ~80% of the total L-VGCC expression, and $Ca_V1.3$. While these subtypes have unique subcellular distributions [30], distinct electrophysiological properties [31], and have been implicated in different physiological roles [32], they cannot be differentiated by pharmacological agents. Thus, one approach that has been used to distinguish between the roles of these two L-VGCC subtypes is to generate transgenic mice lacking either $Ca_V1.2$ or $Ca_V1.3$. Previous work from our lab has suggested that the age-related increase in the sAHP is predominated by $Ca_V1.3$ because genetic deletion of $Ca_V1.3$ significantly reduces the magnitude of the sAHP [16,33], whereas deletion of $Ca_V1.2$ does not appear to impact the sAHP [16]. Researchers have also used molecular and biochemical techniques to elucidate the contribution of the L-VGCC subtypes to the observed age-related increase in calcium current and channel density. The majority of work, which has been performed in the hippocampus of rats, has revealed an increase in the amount of $Ca_V1.3$ mRNA [34,35] and/or protein [36,37], although recent evidence suggests that surface levels [38] and/or phosphorylation [38,39] of $Ca_V1.2$ may be increased in some hippocampal subfields. However, single-cell analysis using reverse transcription polymerase chain reaction (RT-PCR) showed that the magnitude of the increase in $Ca_V1.3$ mRNA from individual hippocampal neurons correlated with the magnitude of the recorded calcium current [34], and additionally, the increase in $Ca_V1.3$ protein expression has been shown to be inversely correlated with the degree of impairment in the Morris water maze [37]. Further, aged mice, unlike aged rats, do not exhibit increased phosphorylation of $Ca_V1.2$ (at serine-1928), which can increase the calcium current mediated by this channel, nor do they exhibit changes in expression levels of $Ca_V1.2$ [27].

Taken together, the available evidence, especially in mice, strongly suggests that an increase in $Ca_V1.3$ is a primary contributor to the age-related dysregulation of neuronal calcium that underlies brain aging. In light of these observations, we have designed a novel line of transgenic mice that are engineered to over-express $Ca_V1.3$

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