

How Genomic Analysis is Changing the Theory of Stress and Aging

The process of aging, biologically speaking, is a highly complicated operation. Although research over the past several decades has helped to elucidate many of these workings, discovering all of the molecular and cellular mechanisms that go into producing the phenotypic trait of aging has proven to be difficult. From a broad perspective, an aged human shows signs of skeletal degradation leading to shortening of stature and weakening bones, muscular atrophy which can result in both heart failure and declining overall strength, slowed metabolism causing problems with digestion or other processes of waste removal, and, possibly worst of all, cognitive decline displayed by a fading memory and a waning ability to process information (Pelland, 2006; AARP.org, 2008). Individuals also show an increased vulnerability to several diseases including neurodegenerative diseases (Magalhaes, 2005). These characteristics of aging have become further described and detailed through experimentation and longitudinal studies; however, the most important contributions of recent studies has been the progress made in identifying the causes underlying these age-related behavioral and physiological trends, in particular the mechanisms underlying brain aging.

As a person ages, several events become enacted within the individual's brain (Pelland, 2006). Overall brain size often begins to decrease especially in the frontal lobe, which is responsible for higher cognitive functions, and the hippocampus, which has significant role in memory storage. The outer layer of the cortex becomes emaciated; this is often related to a lessening of connections between neurons of different brain regions. The myelin that serves as an insulator for action potentials through axons begins to deteriorate, and the communication system of the brain also begins to fail. These structural alterations that take place in the brain explain why people's mental status often becomes languid overtime. These descriptions,

however, still only delineate the large-scale changes that occur as one gets older. To fully understand what is occurring in the brain, researchers had to begin looking at more microscopic levels.

When it is stated that the entire brain begins to shrink, this does not mean that the size of the brain just begins to contract in a fashion one would envision if a shrinkray were directed at it. It does not just become reduced in perfect proportions. Instead, saying the brain shrinks must mean something such as the proteins that make up the brain tissue begin to be enzymatically degraded at a faster rate than they are formed, the structural compounds that help maintain its shape begin to fail, or the individual neurons begin to wither because their ability to take in nutrients begins to decline. In the case of normal human aging, studies have shown that the reasons the brain shrinks, the cortex thins, white matter decreases, and neurotransmitter concentrations diminish are immense. A review in Bioarrays, outlines several, more specific, molecular biomarkers of aging. These include atrophy of pyramidal neurons in the hippocampus, declination in synaptogenesis, decrease in dopamine receptors, reactive glial cells, cytoskeletal abnormalities, increase in genome instability, and increase in susceptibility of neurons to damage by free radicals and inflammation (Erraji-Benchekroun et al, 2007).

Another groundbreaking study recently brought to the forefront another very profound biomarker of aging that significantly contributes to the understanding of this seemingly inevitable phenomenon (Epel et al, 2004). An important feature of life consists of the cell's ability to continue to grow and differentiate. As the body's cells divide, they replace other damaged cells that could impede important biological processes. The cell's ability to divide is largely dictated by the presence and permanence of chromosomes. The main constituent of chromosomal stability is a telomere. These DNA-protein complexes assemble at the ends of

chromosomes and cannot be replicated by standard DNA polymerases; instead, they must be added by telomerase. As an individual ages, their telomeres become progressively shortened with every DNA replication until finally the cell can no longer divide. The length of the telomeres therefore correlates with the time left before the cell becomes arrested in senescence. This obviously also means the length of the telomeres correlates with the biological age of the person because stopping production of new cells means the end of a vital life process.

To further our understanding of aging, it is important for theoreticians to propose what it is that fulfills the criteria for something to cause aging. Therefore, the last question that needs to be answered is: what is it about living that causes a gradual declination in the ability to live? In other words, what is it about metabolism that decreases the body's ability to perform metabolic operations? This is a very complicated question. Most people assume that all things just naturally have a gradual loss of the ability to execute vital functions; however, there must be something that causes the decline. For instance, a car does not just stop working *because* it is fifty years old; it stops working because throughout those fifty years it built up gunk in the pistons, or the nuts and bolts that make up the frame of the engine get worn down by rubbing against other parts. This example attempts to explain the question previously posed and also illuminates a curiously paradoxical idea. Aging must be something that occurs as the byproduct of the necessary processes of life. By thinking in this fashion, researchers are better able to direct themselves along the appropriate path that leads to identifying the cause of the cellular and neuronal destruction brought on by living. Of all the many possibilities, stress is a very likely culprit.

Throughout life humans are constantly being exposed to stress, so it easily fulfills the position of a potential cause of aging. A broad definition of stress is anything perceived as an

adverse stimulus on the body. As research began to uncover the impact of stress, study after study revealed that there is an intimate relationship between stress and aging, particularly brain aging. The general theory states that as an organism becomes exposed to more and more stressors, its body begins to show more signs of aging. The proposal that stress could accelerate the accumulation of certain aspects of aging was first proposed by Hans Selye (1970). Since his broad studies on the stress response, many more researchers have begun investigations of the impact of anxiety on the process of aging. The most extensive amount of research involving stress that has been done examined its effects on hippocampal neurons whose atrophy is considered one of the main byproducts of aging. The original study indicating a positive correlation between elevated levels of corticosterone, the human-glucocorticoid equivalent in rat's, and age-related changes in hippocampal neurons (Landfield, Waymire & Lynch, 1978) sparked a shockwave of studies connecting glucocorticoids, the main hormone released during the stress response, and age-dependent pathologies.

The hippocampus became a prime examinant of aging for several reasons including its role in memory, which progressively declines with age, and its susceptibility to age-related neurodegenerative disorders (Landfield et al., 2007). Once these facts became established, it was only a matter of time before researchers began looking into how the hippocampus changes due to stress. One of the first findings displaying a strong relation between hippocampal neurons and glucocorticoids was that these neurons have a higher density of glucocorticoid receptors indicating that there is a higher receptiveness in hippocampal neurons for the adverse effects of glucocorticoids. The hippocampus also serves as one of the main regulators of the hypothalamic-pituitary-adrenal axis, the axis responsible for generating the stress response. Since the hippocampus provides necessary negative feedback to the HPA axis, the very thing

that the hippocampus attempts to regulate becomes the source of its demise. This almost makes it seem like the hippocampus's job is futile, in that, even normal levels of glucocorticoids can cause age-related degeneration which only leads to more.

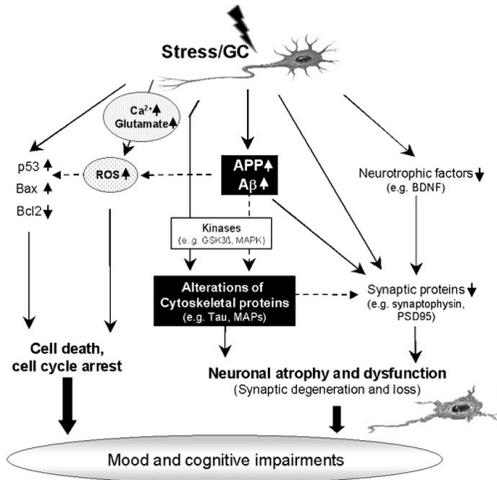


Figure 1: This schematic shows a number of different pathways initiated by glucocorticoids that lead to similar biomarkers as aging (Sotiropoulos, 2008).

This evidence therefore supports the glucocorticoid cascade hypothesis which serves as a theory of how glucocorticoids cause the brain to age. This theory has been further supported by other research which shows that glucocorticoidal damage produces the same biomarkers produced by normal aging. Some of the results of these

other studies show that glucocorticoids prevent synaptogenesis, which explains the thinning of the cortex; glucocorticoids modulate neurogenesis, the hippocampus is

one of the few areas where neurogenesis occurs; hippocampal and frontal cortical volumes decrease as a result of chronic glucocorticoids; and finally glucocorticoids also increase the cells susceptibility to neuronal insults, especially oxidative stress (Landfield et al., 2007; Sotiropoulos, 2008).

In the same study Epel et al outlined the importance of telomeres (2004), the impact of stress on telomere length and telomerase activity was also addressed. By examining 58 mothers who were either caretakers for severely debilitated children or part of a control with healthy children, Epel et al was able to compare both the telomere length in the face of environmental stress and also perceived stress. Her findings showed that the number of years as a caregiver correlated with telomere length and telomerase activity; the more years, the shorter the length and the less activity. Unexpectedly, they also discovered that perceived stress as indicated by a

questionnaire also correlated with length and activity in the same fashion. Although this experiment did not directly test if glucocorticoids had an effect on telomere length, it would not be far-fetched to assume that relative glucocorticoid levels were elevated in proportion to perceived stress. This does need further testing; however, this study does not directly refute the glucocorticoid cascade hypothesis and still concludes that stress has an accelerating effect on the aging process.

The glucocorticoid theory has also had many implications for age-related neurodegenerative disorders, particularly Alzheimer's disease. The characteristic neuropathological features of Alzheimer's are neurofibrillary tangles caused by tau hyperphosphorylation and senile plaques formed by the aggregation of amyloidogenic $A\beta_{42}$ peptides (Alzheimer's Association). For one, glucocorticoids cause similar kinds of damage to the hippocampus and prefrontal cortex as is seen in Alzheimer's. There are also many studies that show glucocorticoids have a direct influence on Alzheimer-related pathological pathways. In 2005, Kulstad et al demonstrated that glucocorticoids have a reducing effect on the concentrations of insulin degrading enzymes, which have been shown to be a major source of the degradation of $A\beta$. By using a variety of methods performed on transgenic mouse models, Green et al. (2006) showed that glucocorticoids increase the production of $A\beta$ by causing transcription-mediated increases in both APP, the precursor protein that leads to $A\beta$, as well as BACE, the enzyme that cleaves APP to form $A\beta$. Other ways glucocorticoids manipulate the progression of Alzheimer's are by increasing its own levels by degrading negative feedback regions of the brain, impairing axonal transport, and reducing neurotrophic factors (Dhikav & Anand, 2007).

As promising as all of these studies seem to be in supporting the glucocorticoid hypothesis, there are many studies that often get dismissed even though their results are important and may imply that the glucocorticoid hypothesis needs revision (Landfield, 2007). Landfield outlines the results of three studies that were inconsistent with the original glucocorticoid hypothesis. For one, corticosterone had an inverse relationship with astrocyte reactivity, a consistent correlate with hippocampal aging. Another set of studies showed that glucocorticoids had an improved effect on memory consolidation, which opposes the fact fading memory is often associated with aging. Third, glucocorticoids were increased in models for caloric restriction, which causes a decrease in many brain biomarkers. These studies give fair reason to assume that in some ways glucocorticoids oppose the aging process.

Given the controversies over which biomarkers actually correlate with aging, it became extremely important to identify more correlates to establish definitively what occurred during the aging process and to reconcile the opposing results for the glucocorticoid hypothesis. This is where genomic analysis became immeasurably essential. Using new gene microarray technologies, it became possible to study a multitude of genes simultaneously to infer what changes occurred through time and also what occurred in different cell types (Erraji-Benchekroun et al., 2007; Landfield et al., 2007).

Using gene microarray expression profiling, Landfield et al. studied changes that occur throughout hippocampal aging in a way that could not be done prior to genomic sciences (2007). By studying thousands of genes over time, Landfield et al was able to uncover a vast distinctive set of biomarkers that reliably change with age. The results of these studies investigating the relative up regulation or down regulation of different genes through normal hippocampal aging could be compared to the relative changes that take place due to glucocorticoidal effects on the

hippocampus. In this fashion, the glucocorticoid theory of brain aging could be further divulged and substantiated.

When Landfield et al. examined the changes in gene expression during normal aging, they discovered that genes that became down regulated often were associated with neuronal processes whereas those that were up regulated were associated with glial processes (2007). Finally using this data set, Landfield et al. could perform a similar microarray study of glucocorticoidal effects on the hippocampal genes. The results proved to be quite paradoxical when viewed in light of the standard glucocorticoid hypothesis. They found that more genes were regulated by both aging and glucocorticoids than chance would suggest; however, they discovered that most of the genes were regulated in opposite directions. This directly conflicts with the original glucocorticoid hypothesis which suggests that aging and glucocorticoids work to produce similar biomarkers in the brain. Upon further investigation, Landfield et al. found that for each of the four possible ways in which genes could be regulated relative to the two types of regulations there seemed to be very distinct sets of cells and functions being impacted. This change in the efficacy of glucocorticoids to affect certain cells, i.e. increasing efficacy when regulated in the same direction and decreasing efficacy when opposite, had many implications as discussed in the paper. These results explained many of the contradictory results of some of the studies inconsistent with the original hypothesis and showed that aging selectively regulates the impact of glucocorticoids according to cell type.

The results of this study still concede that glucocorticoids play a vital role in brain aging; however, the original hypothesis is clearly wrong, or incomplete. By using genomic analysis, Landfield et al. was able to improve our understanding of brain aging and further move us toward a firmer and more comprehensive theory.

New Model of GC Interactions with Aging
 Aging Alters GC Efficacy with Cell-Type and Process Specificity

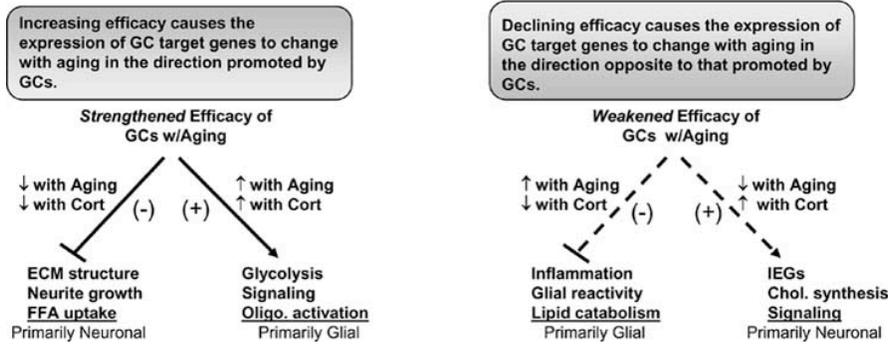


Figure 2 This figure outlines the new glucocorticoid hypothesis of brain aging and includes both aging-dependent changes and relative changes in glucocorticoid efficacy on different cell types (Landfield et al., 2007).

Reference:

Alzheimer's Association, *2008 Alzheimer's Disease Facts and Figures*, (2008)

Dhikav, V. & Anand, K. (2007) Glucocorticoids may initiate Alzheimer's disease: A Potential therapeutic role for mifepristone (RU-486). *Medical Hypotheses*, Vol. 68, pages 1088-1092.

Epel, E., Blackburn, E., Lin, J., Dhabhar, F., Adler, N., Morrow, J., & Cawthon, R. (2004) Accelerated telomere shortening in response to life stress. *PNAS*, Vol. 101, pages 17312-17315.

Erraji-Benchekroun, L., Arango, V., Mann, J. J., & Underwood, M. D. (2007) Genomics to Identify Biomarkers of Normal Brain Aging. *Bioarrays*, pages 83-92.

Green K. N., Billings L. M., Roozendaal B., McGaugh J. L. & LaFerla F. M. (2006) Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *J. Neurosci*, Vol. 26, pages 9047-9056.

"How Aging Changes the Brain." [AARP.org](http://www.aarp.org/health/brain/aging/how_aging_changing_the_brai.html). Staying Sharp. 1 Dec. 2008
<http://www.aarp.org/health/brain/aging/how_aging_changing_the_brai.html>.

[Infoaging.org](http://websites.afar.org/site/PageServer?pagename=IA_bio_h). American Federation for Aging Research. (2008)
<http://websites.afar.org/site/PageServer?pagename=IA_bio_h>

Kulstad, J., McMillan, P., Leverenz, J., Cook, D., Green, P., Peskind, E., Wilkinson, C., Farris, W., Mehta, P., & Craft, S. (2005) Effects of Chronic Glucocorticoid Administration on Insulin-Degrading Enzyme and Amyloid-Beta Peptide in the Aged Macaque. *J Neuropathol Exp Neurol*, Vol. 64, pages 139-146.

Landfield, P.W., Blalock, E. M., Chen, K., & Porter, N. (2007) A New Glucocorticoid Hypothesis of Brain Aging: Implications for Alzheimer's Disease. *Current Alzheimer's Research*, Vol. 4, pages 205-212.

Landfield, P. W., Waymire, J. C., & Lynch, G. (1978) Hippocampal aging and adrenocorticoids: quantitative correlations. *Science*, Vol. 202, pages 1098-1102.

Magalhaes, J. [Senescence.info](http://www.senescence.info). (2005) <<http://www.senescence.info>>

Pelland, M. "How Aging Changes the Body." 21 Aug. 2006. 1 Dec. 2008
<http://aginggrandparents.suite101.com/article.cfm/how_age_changes_your_body>

Selye, H. (1970). Stress and aging. *Journal of the American Geriatric Society*, Vol. 18, pages 669-680.

Sotiropoulos, I., Cerqueira, J. J., Catania, C., Takashima, A., Sousa, N., & Almeida, O. (2008) Stress and glucocorticoid footprints in the brain: The path from depression to Alzheimer's disease. *Neuroscience and Biobehavioral Reviews*, Vol. 32, pages 1161-1173.