

AGE-RELATED CHANGES IN HEAT SHOCK PROTEIN EXPRESSION IN THE MOUSE
HIPPOCAMPUS

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Introduction

Neuroprotection by chaperone proteins is an important part of maintaining a healthy neural network and staving off neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's disease (Mattson, 2000). A nascent protein has all the information required in order to fold into a functional, three dimensional shape. However, the crowded intracellular environment promotes misfolding and aggregation. Organisms have developed chaperone proteins to aid the cell in dealing with these inappropriate interactions (Gragerov et al., 1991; Muchowski and Wacker, 2005). A key class of molecular chaperones includes the heat shock proteins (Hsps). Hsps are involved in a molecular defense against proteotoxic stress and in suppressing the accumulation and aggregation of misfolded or damaged proteins in response to heat, cold, inflammation, anoxia, ischemia, and other acute and chronic cellular stressors (Olsson et al., 2004).

There are five conserved classes of Hsps named for their size in kilodaltons-Hsp100s, Hsp90s, Hsp70s, Hsp60s, and small heat shock proteins. All of these classes of Hsps interact with a wide range of unfolded or damaged proteins (Sharma et al., 2008). Binding to an abnormally folded protein occurs through several mechanisms relying on regulating Hsp affinity for its target protein by the binding and hydrolysis of ATP (Richter et al., 2010). These may include binding to hydrophobic patches, specific sequences, or structural elements. Hsps do not typically aid in folding through contribution of folding information, rather they prevent deleterious intermolecular interactions.

One of the most conserved and widely studied classes of Hsps is the Hsp70 group. Under normal conditions, Hsp70s are involved in *de novo* folding of proteins. When the cell encounters stress, they are involved in the prevention of protein aggregation and the refolding of damaged proteins (Mayer and Bukau, 2005; Zhu et al., 1996). Hsp70s consist of two functional units- a

substrate binding domain, and an ATPase domain involved in substrate exchange (Zhu et al., 1996). The expression and activity of Hsp70s are regulated by cofactors. The most common of these cofactors is the J protein, which brings abnormally folded proteins to Hsp70. The J-domain of this cofactor interacts with the ATPase domain of the Hsp to stimulate hydrolysis of the bound ATP (Kampinga and Craig, 2010; Richter et al., 2010). The binding/hydrolysis of ATP determines the affinity of the chaperone to its substrate.

The Hsp90s class of chaperones is present at high concentrations in the cytosol under normal conditions; under stress, it is further upregulated (Welch and Feramisco, 1982). There are several ways in which the Hsp90s differ from the Hsp70s. They seem to be more specific in their substrate binding. They do not bind unfolded proteins, but more 'native-like' proteins. Also, they evolved from a single component system in prokaryotes to a complicated and sophisticated machinery consisting of a large array of co-chaperones (Richter et al., 2010).

The Hsp100s class includes a conserved family of ATPases that are homologous to the Clp family of proteins in bacteria. They are composed of a hexameric polypeptide with a central pore, through which misfolded proteins are pulled through. After passing through this pore, they are thought to be refolded (Schaupp et al., 2007; Wendler et al., 2009). The exact mechanism of folding is still unknown. The Hsp100s aid in disaggregation of misfolded aggregates by extracting the non-native proteins and refolding them into their native shape (Goloubinoff, 1999).

The small Hsps are poorly conserved and heterogeneous in structure, however they consistently function by binding to and holding partially folded proteins to prevent their aggregation in response to stress. In contrast to the larger Hsps that bind through the binding and hydrolysis of ATP, small Hsps bind to their substrate mainly through hydrophobic interactions

(Lee et al., 2006; Walters et al., 1998). This small Hsp-substrate interaction is maintained until the larger, ATP dependent Hsp's aid in the refolding protein (Richter et al., 2010).

Alzheimer's disease (AD) is a particularly destructive neurodegenerative disease that involves two main proteins, amyloid peptide and Tau protein. The dysfunction of these proteins results in amyloid-beta ($A\beta$) aggregates and neurofibrillary tangles leading to neuronal dysfunction, synapse loss, and clinical dementia (Kumar et al., 2007). Amyloid peptide in the random coil configuration has not been found to be associated with neuronal loss, while neuronal cell death, dystrophic neuritis, and activated microglia are often found in conjunction with concentrated aggregates of the beta sheet configuration (Holcomb et al., 2000). $A\beta$ is processed from amyloid precursor protein (APP); a membrane bound protein whose function is currently unclear (LaFerla et al., 1996). APP is cleaved by several enzymes, commonly β and γ -secretase, to generate peptides of various lengths. $A\beta_{1-42}$ is a hydrophobic product of APP cleavage that is most commonly associated with AD pathology (Murphy and LeVine, 2010). Tau protein is involved in stabilizing the microtubules responsible for macromolecular axonal transport in a healthy neuron. In a diseased neuron, tau becomes hyperphosphorylated and dissociates from the microtubules, causing toxic neurofibrillary tangles (Eroglu et al., 2010; Jinwal et al., 2010a; Jinwal et al., 2010b). Heat shock proteins have been shown to be involved in the prevention of these protein related pathologies by aiding in the proper folding of amyloid and tau.

Kumar and colleagues (Kumar et al., 2007) studied the involvement of Hsp70 and one of its co-chaperone C-terminus Hsp70 interacting protein (CHIP), in the processing of APP into $A\beta$. In the presence of a proteasome inhibitor, APP levels increased, as did CHIP, suggesting that CHIP is involved in the proteasomal clearance of APP. Under these conditions, Hsp70 also increased, and Hsp70-CHIP-APP complexes were detected by immunoprecipitation. In neuroblastoma culture, CHIP and Hsp70 overexpression by inducible adenoviral construct, either

alone or synergistically, protected cells from A β stress. Hsp70, Hsp90, and CHIP overexpression also prevented A β related toxicity in primary neurons by a reduction in A β levels.

The link between AD and Hsp110 was studied by using an Hsp110 knockout mouse (Eroglu et al., 2010). They show that the loss of Hsp110 function causes an age-dependent increase in phosphorylated tau. These mice also exhibit AD related pathologies, such as the appearance of neurofibrillary tangles and A β plaques. In immunoprecipitation assays, the authors showed that Hsp110 interacts with glycogen synthase kinase-3 β , a kinase that phosphorylates Tau, and PP2A, a protein phosphatase that desphosphorylates Tau. These findings indicate that the role of Hsp110 is critical for managing the folding environment required for the proper phosphorylation and dephosphorylation of Tau.

In a recent study, Jinwal and colleagues, looked at the involvement of the constitutive form of Hsp70, (Hsc70), in the stabilization of hyperphosphorylated Tau protein after microtubule destabilization (Jinwal et al., 2010a). They hypothesized that, since the Hsp70 family of chaperones is known to interact with Tau protein, and Hsc70 is in much higher abundance than Hsp70, Hsc70 must be highly involved in Tau processing, especially after its dissociation from the microtubules. HeLa cells transfected with human Tau were lysed and the Tau protein levels were quantified via Western blot. In *Xenopus* oocyte extracts, Hsc70 supplemented Tau protein, increasing microtubule formation under all experimental conditions, as shown by fluorescence microscopy. Using nocodazole to disrupt and destabilized the microtubules, they were able to show that Hsc70 and Tau interaction increased after microtubule destabilization by immunofluorescent confocal microscopy as well as immunoprecipitation. Hyperphosphorylated Tau has been repeatedly correlated with AD tangle pathology. In a hyperphosphorylating environment with an abundance of GSK β , a kinase known to phosphorylate Tau at several locations, Hsc70-Tau interaction increased greatly. Hsc70 held

hyperphosphorylated Tau in a more linear conformation, instead of allowing it to fold into the MC1 formation that is an early pathological formation of Tau in AD. This binding is speculated by the authors to actually do more harm than good by inadvertently preserving the levels of hyperphosphorylated Tau in the cell.

Hsp70 and Hsp90 have also been implicated in the prevention of A β aggregate formation (Evans et al., 2006). Using turbidity assays, they showed that with increasing concentrations of Hsp70, A β aggregation was inhibited resulting in decreased turbidity and lower absorbance. Increasing concentrations of Hsp70 decreased the signal of thioflavin T, a fluorescent marker for A β aggregates. Hsp70 also counteracted the formation of A β aggregates as seen in electron micrographs. The anti-A β of HSP70 activity is increased by co-chaperone Hsp40 in additional thioflavin T assays. Very similar results were seen in experiments using Hsp90 to inhibit formation of A β aggregates.

Hsp90 has been shown to be involved in the clearance of exogenous A β in the rat hippocampus (Takata et al., 2003). The premise of their study is based on microglial activation in an anti-A β manner. One of the brain's methods for clearing A β is through phagocytosis by microglia, the resident phagocytic cell of the brain. Takata and colleagues previously showed that extracellular Hsp's 90, 70, and 32 may facilitate A β clearance by the activation of microglial phagocytosis (Takata et al., 2007). In their previous studies, Hsp90 was increased in AD brains, and colocalized with amyloid plaques. These findings led them to examine the effect of Hsp90 in an *in vivo* rat model of A β deposit formation. Mixtures of A β ₁₋₄₂ and Hsp90 as well as A β ₁₋₄₂ alone were injected into the hippocampus, and quantified using immunohistochemistry. An enzyme-linked immunosorbent assay (ELISA) was performed for A β ₁₋₄₂, as well as the inflammatory markers tumor necrosis factor (TNF)- α , and interleukin (IL)-6. Simultaneous

injection of A β ₁₋₄₂ with Hsp90 significantly reduced the amount of A β ₁₋₄₂ at 14 days post-injection. The levels of cytokines were also increased when A β was coinjected with Hsp90.

The propensity for the elderly to develop AD begs the question, what happens to the brain's ability to clear protein aggregates as we age? Levels of inducible Hsp70 in the inferior colliculus of aged rats were found to be 56.5% less than that of 3-month olds (Helfert et al., 2002). Increased age brings increased cellular stress through reactive oxygen species and the resulting oxidative damage to the cell (Golden et al., 2002). To combat this, there is an increase in Hsp72 expression with age in the substantia nigra, hippocampus, and cerebellum, as well as increases in Hsc70 (Calabrese et al., 2004). In several brain regions, including the inferior colliculus and cerebellum, the expression of Hsp72 in aged mice was less than half that of young mice in response to acoustic stress (Helfert et al., 2002). mRNA levels for Hsp70 were also shown to be decreased in 24 month old rats when compared to 5 month old rats after a 90 minute heat shock (Blake et al., 1991).

An important brain region involved in AD is the hippocampus. This region is involved in spatial learning and memory, and is one of the first areas to experience cell death in AD patients, with an exceptional amount of cell death in the CA1 pyramidal neurons (Pardue et al., 1992). It was once thought that hippocampal cell death was a part of the normal aging process, however stereological approaches to several species, including humans, have led to the conclusion that neuronal cell death is not involved in normal aging (Morrison and Hof, 1997).

Pardue and colleagues studied the ability of aged rats to induce Hsp expression in the hippocampus in response to stress (Pardue et al., 1992). Using thermal stress to induce Hsp70, and *in situ* hybridization to quantify Hsp70 mRNA, they found that the relative expression levels of Hsp70 mRNA were several fold lower than the young animal, especially in the dentate gyrus of the hippocampus. These findings suggest that some populations of neurons in the

hippocampus of the aged rat may be at increased risk of stress-related damage due to the inability to upregulate Hsps.

During amnesic mild cognitive impairment (aMCI), a pre-dementia stage between normal aging and AD, Di Domenico and colleagues showed that Hsp's 27, 32, 60, 70, and 90 in the hippocampus, parietal lobe, and cerebellum generally show only a mild induction (Di Domenico et al., 2010). This weak cell stress response allows for oxidative stress and protein modification, leading to a favorable environment for the progression from aMCI to AD. This study suggests that neurons don't induce chaperone protection, at least to the degree seen in other studies, until AD fully develops with full presentation of plaques and tangles.

These data lead us to hypothesize that aged mice have an increased basal level of Hsp's in response to the cellular stress of aging, while having a reduced ability to upregulate additional Hsp expression in the event of additional thermal stress. This inability to upregulate Hsp's to circumvent stress-related protein damage would suggest a higher susceptibility to protein related pathologies, such as those involved in AD. Given the importance of the hippocampus in learning and memory, and its early and severe destruction during AD pathology, we have chosen this brain region as the focus of our study. In this study, we have comparatively quantified three AD relevant Hsp's in three age groups of mice to better understand how their expression changes with age, both constitutively and in the event of an acute stress.

Methods

Subjects

Male C57BL/6J mice from the TCU vivarium will be utilized in all experiments. All animals will be housed and treated in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), and in accordance with protocols

approved by the Institutional Animal Care and Use Committee (IACUC) of Texas Christian University.

All subjects will be housed in groups of three or four in standard cages (12.5cm x 15cm x 25cm). All experimental groups and control groups will be on the same light schedule, lights on at 0600 and lights off at 1800, and both food and water will be available *ad libitum*.

Treatment groups and heat shock

Treatment groups were organized by age. The young group consisted of mice ranging in age from 4-6 weeks. The middle aged group ranged from 8-10 months of age, and the old group ranged from 18-24 months of age. Each group consisted of 15 mice. Average basal protein expression for each group was determined using the average of four untreated mice of each age group.

In order to induce a stress response, mice were anesthetized with ketamine and xylazine, and heated under a heat lamp to a colonic temperature of 39°C, measured by a rectal thermometer (Thermalert TH-5, Physitemp, Clifton, NJ). After this temperature had been maintained for 15 minutes, mice were allowed to recover on the cool, metal operating table, and then replaced into their home cages. Twenty-four hours after heat shock, mice were euthanized by CO₂ asphyxiation, brains removed, and hippocampus dissected. Removed tissues were homogenized in a lysis buffer consisting of 25mM Tris @ pH 7.5, 150mM NaCl, 1% Nonidet-P40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate, and 1x protease inhibitor (IBI Scientific, Peosta, IA) and allowed to lyse and freeze overnight.

Tissue Preparation

Protein concentration was determined by DC protein assay, which utilizes a reagent that is commonly used with detergent-based lysis buffers. A 1.52µg/µl protein standard serially diluted in lysis buffer down to 0.2µg/µl provided the standard curve with which to determine the

concentration of the unknowns. 5 μ l of unknown protein were pipetted into a 96-well plate, followed by the addition of 25 μ l of reagent A' and 200 μ l of reagent B. After 15 minutes of incubation in the dark, the absorbance of each well was read at 750nm in the plate reader (BMG LabTech FLUOstar Omega, Cary, NC).

Western Blots

After determining the concentration of the stock solutions of protein, samples were prepared for loading by mixing proper amounts of protein sample, lysis buffer, and sample buffer to equal a concentration of 1 μ g/ μ l. Proteins were denatured by boiling for 5 minutes in a dry bath (Labnet Intl., Woodbridge, NJ), with as the presence of β -mercaptoethanol (Sigma, St. Louis, MO) in the sample buffer to reduce intramolecular disulphide bonds.

Boiled samples were loaded into 15-8% gradient acrylamide gels (Bio-Rad, Hercules, CA) at 25 μ l per well. All Western blot hardware, including gels, gel cassettes, gel box, and transfer cells, is a product of Bio-Rad, Hercules, CA. In the case of CHIP, 12.5% gels were used to achieve a better separation of the smaller proteins to allow us to also probe for similarly-sized β -actin. Current was applied to the gels at 200 watts for 50 minutes. Proteins were transferred (Bio-Rad Trans-blot SD, Bio-Rad, Hercules, CA) from the polyacrylamide gel electrophoresis (PAGE) gel to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA). The current applied through the transfer cell was conducted through filter papers (Kaysville, UT) soaked in electrolytic buffer as per the instructions provided with our transblotter. The transfer of protein from gel to membrane required 0.15 amps per gel, for 40 minutes.

Success of the transfer was gauged by the results of a Ponceau-S stain (Sigma, St. Louis, MO) to stain nonspecifically for all proteins. This was washed off in PBST (PBS + 0.3% Tween). The membrane was then blocked in 25% non-fat, blotting grade milk in PBST with

0.02% azide (Sigma, St. Louis, MO) for 45 minutes. Primary antibodies, polyclonal goat anti-Hsp70, polyclonal goat anti-Hsc70, polyclonal goat anti-Hsp105, and polyclonal rabbit anti-CHIP were mixed at 1:500 dilution in 25% bovine serum (Amresco, Solon, OH). Monoclonal mouse anti- β -actin was mixed in bovine serum at 1:2500. All primary antibodies are products of Santa Cruz Biotechnology, Santa Cruz, CA. Membranes were incubated in primary antibodies at 4°C overnight.

After the removal of primary antibodies, the membranes were washed in several washes of PBST, followed by the addition of peroxidase-linked secondary antibodies; donkey anti-goat, donkey anti-rabbit, and rabbit anti-mouse, all at concentrations of 1:5000. All secondary antibodies are polyclonal products of Jackson ImmunoResearch, West Grove, PA. Membranes were incubated with secondary antibodies for one hour.

After removal of secondary antibodies, the membranes were washed in several washes of PBS. They were then placed on transparencies and coated in SuperSignal West Pico chemiluminescent substrate (Thermo Scientific, Waltham, MA). An image of the resulting chemiluminescence was acquired using a Syngene G:Box (Syngene, Frederick, MD). Densitometric analysis was done using GeneTools software, with β -actin as the loading control.

Results

Representative Western blot demonstrates basal and inducible HSP expression. All lysates were run on SDS-Page gels to compare the basal levels and inducible levels of HSP expression.

Figure 1 shows one experiment testing for HSP70 expression, and is representative of all HSP comparisons and includes uninduced and induced expression for all three age groups tested.

Densitometry values for each uninduced sample band were compiled and a mean determined.

This mean value was then divided by the mean values for β -actin to obtain a mean ratio for basal

expression. Next, each induced sample was compared to its own β -actin and the mean ratio was then compared to the basal expression to obtain a fold increase.

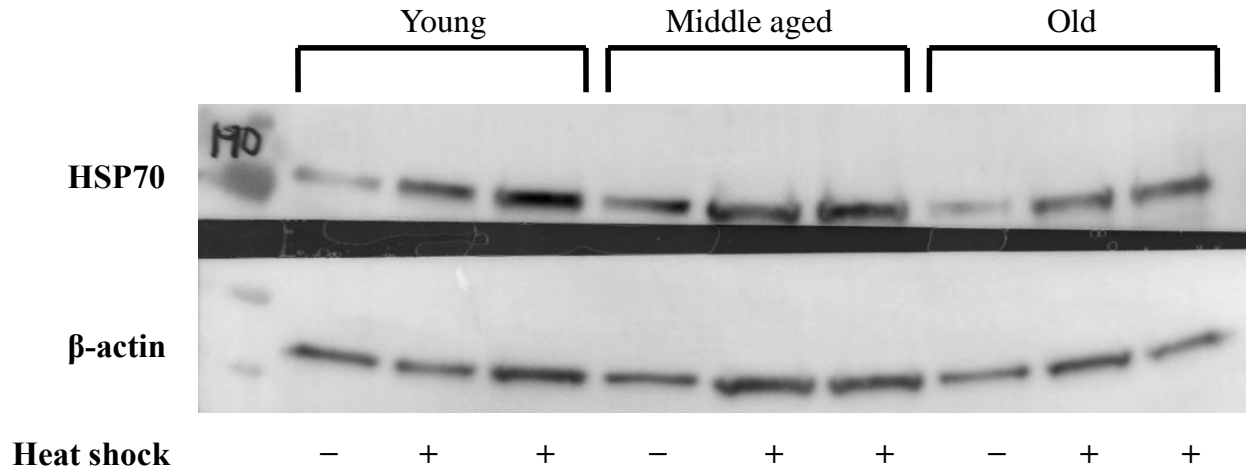


Figure 1. Representative Western blot for HSP70. Young, middle aged and old animals were compared for their basal heat shock level (-) and for their induced level (+) of expression. β -actin is shown as a loading control for each sample.

HSP70 expression at basal and induced levels. Basal levels of HSP 70 relative to β -actin expression seem to increase with age; however this is only a trend and not significant (Fig 2A). After heat shock, HSP70 expression is increased 2.5 fold in young mice, 1.5 fold in middle aged mice, and the aged mice show very little increase in HSP70 expression following acute heat stress (Fig 2B). There is a significantly ($p < 0.05$) reduced ability to induce expression of HSP70 between young and middle aged mice, with an even more pronounced reduction ($p < 0.01$) in HSP70 response when comparing young and old mice ($n=15$).

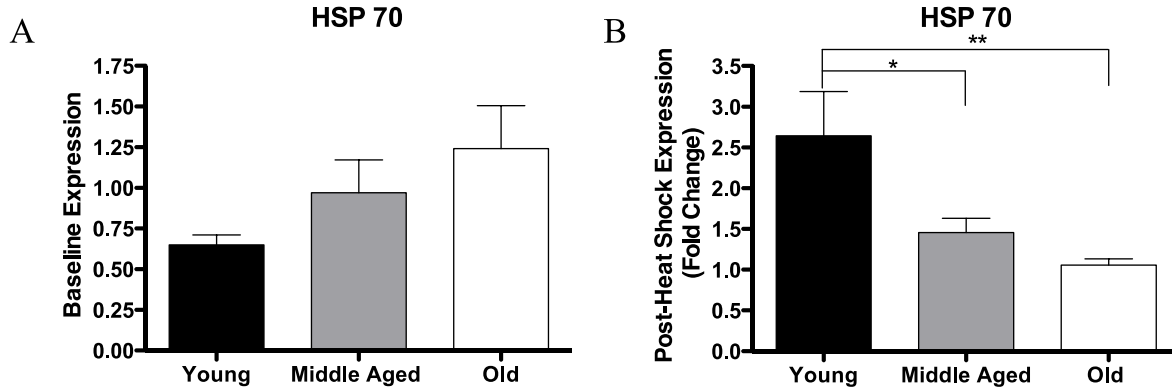


Figure 2. Baseline and inducible HSP expression compared across three age groups. **A.** There is an increase in baseline HSP70 expression with increased age, though not significant. **B.** As age increases, the ability to induce HSP70 expression decreases significantly. * $p < 0.05$, ** $p < 0.01$.

HSP105 expression at basal and induced levels. There is no significant difference in baseline expression of HSP105 between the three age groups of mice (Fig 3A). Following heat stress, middle-aged mice showed a reduction in HSP105 production by ~50%, while young and old mice increased HSP105 in response to heat stress by ~25% (Fig 3B). There is no significant difference between induced levels of HSP105 between young and old mice, however middle -

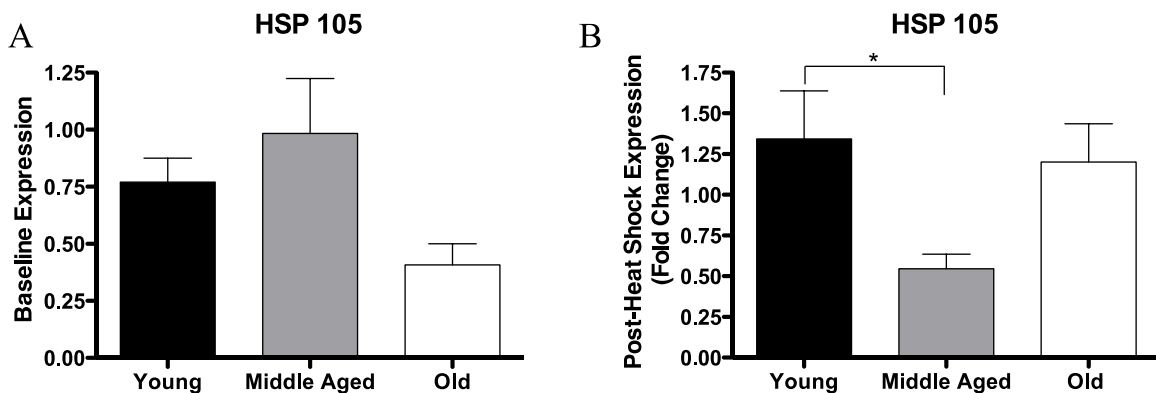


Figure 3. Baseline and inducible HSP expression compared across three age groups. **A.** There is increased baseline expression of HSP105 in middle aged animals when compared to young animals, though not significantly. Old animals showed lower baseline HSP105 expression when compared to young mice, but again this difference was not significant. **B.** Young and old mice showed a strong ability to induce HSP105 expression when compared to middle age, but only young mice showed a significant induction of HSP105. * $p < 0.05$.

aged mice showed a significantly ($p < 0.05$) reduced ability to induce HSP105 expression following heat stress when compared to young mice.

CHIP expression at basal and induced levels. Baseline expression of CHIP increased significantly with age, with differences between young and middle age ($p < 0.01$) and middle and old aged mice ($p < 0.001$) (Fig 3A). The increase in basal CHIP expression between young mice and middle aged mice is over five fold, and almost 25 fold between young and old mice ($p < 0.001$). Following heat shock, there is no significant difference in ability to increase CHIP expression, with all three age groups upregulating CHIP by over 25% (Fig 3B)

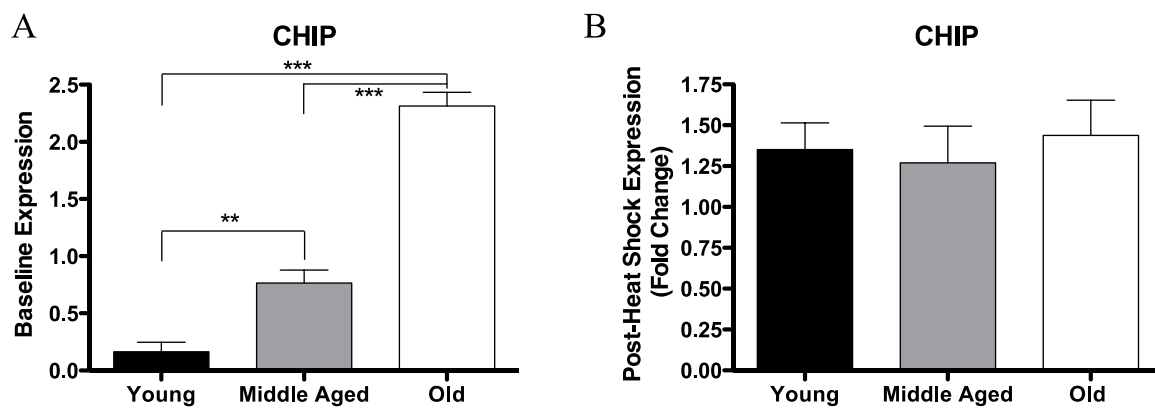


Figure 4. Baseline and inducible HSP expression compared across three age groups. **A.** Baseline expression of CHIP significantly increased with age however there was no difference (**B**) in ability to induce CHIP expression in response to heat shock. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

HSC70 expression at basal and induced levels. There is significantly more expression of basal HSC70 in young mice when compared to middle aged ($p < 0.05$) and old mice ($p < 0.01$) (Fig 5A). Following an acute heat stress, there is no significant difference in ability to upregulate HSC70, with all three age groups increasing HSC70 at least two fold (Fig 5B).

Discussion

As previously shown in other tissues (Blake et al., 1991), the hippocampus shows a reduced ability to induce expression of HSP's in response to an acute stress. Basal levels of

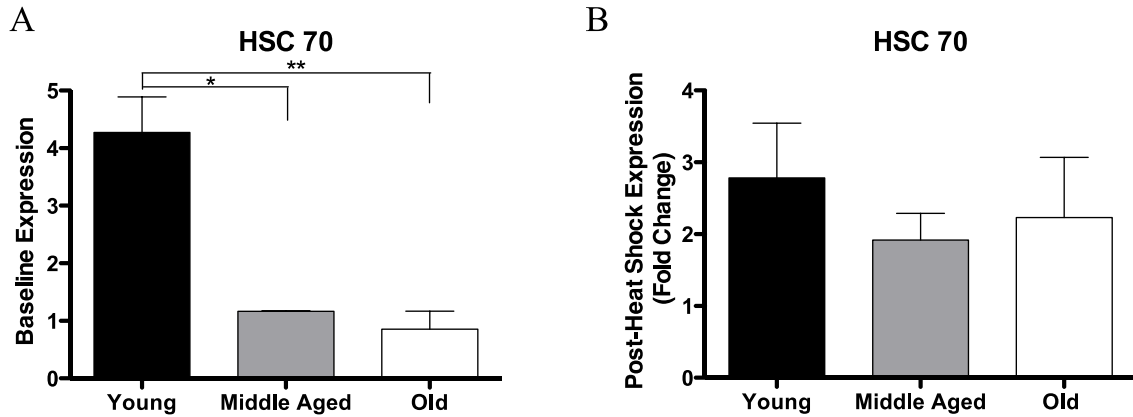


Figure 5. Baseline and inducible HSP expression compared across three age groups. **A.** There is a significant decrease in constitutive HSC70 expression with age, but there was no difference in ability to induce HSC70 in response to heat shock (**B**). * $p < 0.05$, ** $p < 0.01$.

HSP70 seem to trend upward in response to the increased chronic, anoxic, oxidative, cellular stress of aging, though our study did not show this difference to be significant (Fig 1A).

Following heat shock, the young mice showed a comparatively strong (2.5 fold) induction of additional HSP70 to combat the acute stress, while middle aged and old mice were unable to induce expression of HSP70 more than 1.5 fold (Fig 1B). This significant difference could be attributed to the already increased levels of HSP70 in older mice prior to the heat shock, creating a ceiling effect in the amount of expression a mouse is able to induce.

Baseline HSP105 expression follows a similar trend between young and middle aged mice, however expression trends downward again when comparing middle-aged and old mice (Fig 2A). None of these differences are significant. Following heat stress, middle-aged mice show a significantly reduced HSP105 response when compared to young mice. Interestingly, old mice showed a strong ability to induce HSP105 expression, with no significant difference between young and old induced HSP105 expression (Fig 2B). The inability of middle-aged mice to induce HSP105 may be attributed to the higher baseline expression. It also may be possible that HSP105 does not respond to heat stress in the hippocampus in the same way that the other

molecular chaperones measured in this study. Further studies measuring HSP105 may require different cellular stressors, such as inflammation, hypothermia, or exercise.

CHIP expression increased in line with HSP70 with an increase in age, though more significantly (Fig 3A). This is to be expected due to it being a co-chaperone with HSP70. However, following heat stress, all three age groups were able to equally induce CHIP. This suggests that HSP70 and CHIP, while directly related and interacting, may be regulated differently in response to thermal stress. CHIP expression may be very sensitive to hyperthermic stress, and therefore is substantially increased following an acute heat stress.

Baseline HSC70 expression is much higher in young mice when compared to middle aged and old mice. While, our initial hypothesis was that this protein would be expressed at much higher levels in aged mice in order to combat the cellular stressors involved in aging, it is not surprising that HSC70 levels may be lowered with a concurrent increase in the inducible version, HSP70 as seen in Figure 1A. HSC70 is often regarded as a constitutively expressed protein, with levels staying relatively unchanged in response to acute stress. However, our data show a strong and relatively equal induction of this chaperone across all three age groups following heat shock.

One caveat of this study is that not all HSP's are created equally. Because they are a part of a cellular stress response, some HSP's respond differently to different stressors. Some HSP's respond well to one type of stress, and others respond to a variety of stressors. An addition of different types of stressors, such as inflammation, hypothermia, psychological, or drug induced would give a better idea of these animals' abilities to induce a heat shock protein response.

Due to the nature of research on the brain, we do not have the luxury of comparing the levels of HSP's pre-treatment and post-treatment, within the same brain. This, combined with the

nature of HSP's, and their varied expression levels in response to a number of stressors that can be difficult to control for, creates some confounding variables that are almost unavoidable. It is not feasible to know what kind of pre-existing conditions the mice could have that may be affecting their HSP expression, short of doing an autopsy on every test subject.

Lastly, there is the issue of balancing inducible HSP expression with the brainstem's ability to regulate body temperature. There is a fine line between a very stressful event eliciting an HSP response, and a stressful event that will result in the death of the mouse. This is an even more difficult issue regarding the more aged mice, as they are much less healthy and resilient than the younger mice, and thus less able to tolerate such a stressful event as a heat shock. It is difficult to induce a heat stress that is equally stressful for both a young, resilient, healthy mouse, as well as an old mouse that is already battling the struggles of aging. One way to circumvent this problem is to apply a heat stress in a gentler manner, utilizing a heating pad, or a heated chamber, in contrast with the more harsh and direct heat of a heat lamp. In a study done by Tolson et al., a warm water bath was used to induce hyperthermia in anesthetized mice wearing life vests specially weighted to submerge the entire animal except the mouth to allow for breathing (Tolson and Roberts, 2005). This method could provide a much more gentle and survivable application of whole-body thermal stress to the mouse, without putting too much stress on the thermoregulative responsibilities of the brain stem.

Conclusion

These data suggest that the aged mouse, due to higher basal levels of HSP's, particularly HSP 70, is less able to induce a molecular chaperone response in order to rescue and repair damaged proteins in response to an acute stress. HSP 70 is one of the most widely and heavily studied HSP's, and its importance in the stress response is well documented. Protein related pathologies

such as AD are kept relatively under control for our entire lives due in part to molecular chaperones such as HSP70 and CHIP. The loss of expression of these proteins under stress as we age may be a contributing factor to the increased incidence of AD in the elderly.

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Personal Background

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ABSTRACT

AGE-RELATED CHANGES IN HEAT SHOCK PROTEIN EXPRESSION IN THE MOUSE HIPPOCAMPUS

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Heat shock proteins (Hsps) serve as the cell's natural mechanism for circumventing protein misfolding and aggregation often involved in cellular stress. These stressors can lead to neurodegenerative pathologies like Alzheimer's disease (AD). Research suggests that aged animals lose the ability to upregulate Hsps in response to an acute stress, and this dysregulation may contribute to the onset of AD. We hypothesize that aged animals will show elevated Hsp levels due to the increase in cellular stress associated with aging. We further hypothesize that this increased level of Hsps will inhibit the aged animal from further expressing Hsps in the event of an acute stress. We compared basal and inducible brain hippocampus levels of four Hsps previously shown to stabilize two proteins commonly associated with AD, amyloid beta and tau. Our results support our hypothesis that Hsp expression changes with age and may be a contributing factor in AD.