HHS Public Access

Author manuscript

Neurobiol Dis. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

Neurobiol Dis. 2016 January ; 85: 73–80. doi:10.1016/j.nbd.2015.10.014.

Mini-Review: Retarding Aging in Murine Genetic Models of Neurodegeneration

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Abstract

Retardation of aging processes is a plausible approach to delaying the onset or slowing the progression of common neurodegenerative disorders. We review the results of experiments using murine genetic models of Alzheimer disease and Huntington disease to evaluate the effects of retarding aging. While positive results are reported in several of these experiments, there are several discrepancies in behavioral and pathologic outcomes both within and between different experiments. Similarly, different experiments yield varying assessments of potential proximate mechanisms of action of retarding aging. The anti-aging interventions used for some experiments include some that show only modest effects on lifespan, and others that have proven hard to reproduce. Several experiments used aggressive transgenic neurodegenerative disease models that may be less relevant in the context of age-related diseases. The experience with these models and interventions may be useful in designing future experiments assessing anti-aging interventions for disease-modifying treatment of neurodegenerative diseases.

Keywords

Alzheimer disease; Huntington disease; Senescence; Aging; Neurodegeneration; Amyloid; Polyglutamine

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Conflicts of Interest: The authors have no relevant conflicts of interest to declare.

Introduction

Neurodegenerative disorders such as Alzheimer disease (AD), Parkinson disease (PD), Frontotemporal dementias (FTDs), Huntington disease (HD), Amyotrophic Lateral Sclerosis (ALS), and Spinocerebellar Ataxias (SCAs) are common causes of morbidity and premature mortality. Incidence of the most common and debilitating neurodegenerative diseases is strongly age-related. This is true also for the less common Mendelian forms of AD, PD, FTDs, and ALS, as these variants exhibit age-related penetrance. Similarly, HD and SCAs exhibit age-related penetrance. With the number of elderly individuals now rising dramatically in both developed and developing nations, prevalence of neurodegenerative diseases is expected to soar. Efforts to find disease-modifying treatments have been largely unsuccessful. These efforts focus mainly on identifying pathogenic mechanisms specific to each disease process. The relative lack of progress with these approaches and the age-related nature of neurodegenerative disease incidence suggests that modulation of aging per se may be a useful alternative approach for delaying the onset or retarding the progression of neurodegenerative diseases (Taylor and Dillin, 2011; Fontana et al., 2014; Herskovits and Guarante, 2014; Longo et al., 2015). This concept is supported by an impressive body of knowledge identifying interventions - genetic, dietary, and more recently pharmacologic - that profoundly retard aging and its pathophysiological effects in a number of invertebrate and murine model systems (Taylor and Dillin, 2011; Fontana et al., 2014; Herskovits and Guarante, 2014; Longo et al., 2015; Fontana and Partridge, 2015; Harrison et al., 2014; Wilkinson et al., 2012; Miller, 2012; Harrison et al., 2009).

Correlative human data suggests that these model system results are relevant to man. Caloric restriction (CR) consistently retards aging and reduces age-related pathologies in rodents (Fontana and Partridge, 2015). CR in humans reproduces many of the beneficial physiologic changes seen in rodents (Fontana and Partridge, 2015). Humans voluntarily pursuing long-term CR exhibit lower cardiovascular disease and cancer risk factors (Fontana et al., 2010). In C. *elegans* and mice, reduced signaling through a phylogenetically conserved cell stress response pathway mediated by insulin/insulin-like peptide signaling extends lifespan with reduction in age associated pathologies (Kenyon, 2011; Brown-Borg et al., 1996; Flurkey et al., 2001). Some human polymorphisms in genes of this pathway are associated with greater longevity (Bonafe et al., 2003; Suh et al., 2008). Individuals with Laron dwarfism, an autosomal recessive loss of Growth Hormone Receptor function, are characterized by reduced Insulin-like Growth Factor 1 production and show dramatically lower incidence of certain major diseases of aging, including cancer and diabetes (Guevara-Aguirre et al., 2011).

Experiments in invertebrate model systems, notably C. *elegans*, support the concept that retarding aging can significantly decrease neurodegenerative pathologies. These experiments used models with expression of pathogenic proteins such as Amyloid Precursor Protein (APP) peptides or polyglutamine peptides. Genetic interventions known to slow senescence and extend lifespan reduce the deleterious effects of these pathogenic peptides (Morley et al., 2002; Hsu et al., 2003; Cohen et al., 2006). The last 2 decades have witnessed the development of numerous murine genetic models of neurodegenerative disease. These experimental systems supported studies, analogous to those performed in C. *elegans*, to

determine if neurodegenerative pathologies can be modulated by pathways that slow aging and its sequelae in mice. The development of approaches for prevention and treatment of human neurodegenerative disease, based on insights from such preclinical studies, merits attention. Compounds, including some used in clinical practice, have been identified that retard aging in mice. Some of these compounds, such as rapamycin, acarbose, and 17-α-estradiol, may partially mimic the actions of genetic manipulations that retard aging in invertebrate and murine models (Harrison et al., 2014; Wilkinson et al., 2012; Harrison et al., 2009). These agents are plausible candidates for clinical trials in neurodegenerative diseases and should be evaluated in appropriate preclinical experiments. Experiments to evaluate anti-aging strategies for effects on late-life illnesses are expensive and time-consuming. Prior experience with anti-aging interventions in murine genetic models of neurodegeneration should provide valuable lessons for the design of future experiments testing drugs and dietary manipulations for effects on late-life neurodegenerative illnesses in mice. We review this prior experience and suggest experimental design features that may improve the quality of future experiments in this area.

Alzheimer Disease

Murine genetic AD models feature transgenic expression of human mutant Amyloid Precursor Protein and other proteins implicated in the pathogenesis of AD, such as Presenilins or Tau. To date, 7 experiments using some form of aging modulation in mice have been reported (Table 1). These experiments used a variety of AD models, aging interventions, and endpoints.

The initial experiments used CR. Patel et al. (2005) studied the effects of CR on 2 murine genetic AD models: $APP_{swe/ind}$ (J20) mice containing transgenes with 3 pathogenic APP mutations and a line hemizygous for separate transgenes expressing mutant APP or mutant presenilin 1 (mAPP+mPS1) (Mucke et al., 2000; Holcomb et al., 1998). CR was started at 14–15 weeks of age, and mice were euthanized for histopathologic endpoints at 24 weeks. There was evidence that CR reduced amyloid plaque burden in both lines, though measures of amyloid plaque burden were not consistent in these analyses. CR reduced the area occupied by amyloid plaques in cortical but not hippocampal sections of mAPP+mPS1 mice. Fibrillar amyloid, measured by Congo Red staining, was reduced in both cortex and hippocampal formation of mAPP+mPS1 mice. CR reduced amyloid plaque number and size in hippocampal and cortical sections from $APP_{swe/ind}$ mice. In $APP_{swe/ind}$ mice, CR may have reduced reactive astrocytosis, but there was no such effect in mAPP+mPS1 mice. There was no CR effect on APP mRNA levels. Wang et al. (2005) studied CR effects in a transgenic model expressing mutant APP (Tg2576) (Hsiao et al., 1996). CR was initiated at 3 months, and the mice were evaluated at 12 months. CR, as expected, produced lean mice with improved glucose tolerance compared to ad libitum fed Tg2576 mice. Histologic analysis indicated marked reduction in amyloid plaque burden. Levels of the amyloidogenic APP derived peptides Aβ1–40 and Aβ1–42 were reduced in CR animals when measured with ELISA and mass spectrometry, although total APP levels were comparable to controls. Further analysis suggested that CR enhanced non-amyloidogenic pathway processing of APP by an α-secretase activity, possibly by elevating expression of the metalloproteinase ADAM10. CR also increased expression of Insulin Degrading Enzyme (IDE), which may

degrade Aβ peptides. Halagappa et al. (2007) employed both CR and intermittent fasting (every other day feeding, "IF") in a triple transgenic AD model (3xTgAD) expressing mutant APP, mutant Presenilin 1 (PS1), and mutant Tau (Oddo et al., 2003). This group began CR and IF at 3 months with biochemical readouts at 17 months, and with behavioral data collected at 10 and 17 months. CR and IF improved performance in the open field and Morris water maze equivalently at 10 and 17 months. CR reduced Aβ1–40, Aβ1–42, and abnormally phosphorylated Tau levels in the hippocampal formation. IF, in contrast, had identical behavioral effects but no effects either on hippocampal Aβ peptide or on levels of abnormally phosphorylated Tau.

Other groups used crosses between murine genetic AD models and mutant lines thought to exhibit significant retardation of aging. Killick et al. (2009) crossed Tg2576 mice to insulin receptor substrate 2 knockout mice (*Irs2*$^{-/-}$). Mice were evaluated with contextual fear conditioning at 10–12 months age and for histologic and biochemical readouts at 11–14 months of age. Double mutant mice exhibited better performance in a fear conditioning task than Tg2576 mice without the IRS2 deletion. Histologic analysis indicated that the Irs2$^{-/-}$ genotype reduced amyloid plaque burden but did not lead to changes in the concentrations of APP, C-terminal APP fragments, or soluble Aβ1–40 and Aβ1–42 levels. Double mutant mice exhibited lower concentrations of aggregated Aβ1–40 and Aβ1–42. These results suggested that changes in APP processing could not account for differences in amyloid plaque deposition. The expression of 2 proteins implicated in Aβ peptide degradation, transthyretin and membrane bound IDE, was increased in double mutant mice compared to mice carrying the Tg2576 allele alone or wild-type mice. In contrast to the APP results, expression of another potentially toxic protein species, abnormally phosphorylated Tau, was increased in double mutant mice compared to mice carrying the Tg2576 allele only. Freude et al. (2009) performed a similar series of experiments with Tg2576 mice and *Irs2*$^{-/-}$ double mutants. This group reported reduced mortality in female, but not male, double mutants and reduced brain Aβ peptide concentrations at 12, but not 48, weeks of age. Further studies of APP degradation products suggested an alteration in APP processing. Freude et al. (2009) extended their studies in mice with deletion of hippocampal neuronal IGF1 receptors (IGF1Rs; synapsin Cre x floxed IGF1R) crossed to Tg2576 mice. Hippocampal IGF1R knockout rescued early mortality in mice of both sexes and reduced hippocampal Aβ peptide accumulation at 28 weeks of age. Studies of APP degradation products were consistent with alteration of APP processing. Similar experiments with deletion of neuronal insulin receptors or heterozygous deletion of neuronal IGF1Rs had no effects on Tg2576 mortality.

Cohen et al. (2009) also employed reduction of IGF1Rs. This group generated germline IGF1R heterozygotes (IGF1R$^{+/-}$) also expressing a transgene containing mutant APP and PS1 ($APP_{swe}PS1\Delta E9$) (Borchelt et al., 1997). Double mutant mice at ages 11–15 months exhibited improved memory performance in the Morris water maze and better rotorod performance compared to $APP_{swe}PS1\Delta E9$ mice. Histology at 12–13 months revealed less reactive gliosis, neuronal loss, and synaptic loss in double mutant mice than in $APP_{swe}PS1\Delta E9$ mice. Immunohistochemistry revealed similar amyloid plaque burden in double mutant and $APP_{swe}PS1\Delta E9$ mice but further analysis suggested smaller, denser amyloid plaques, a result confirmed by electron microscopy. Biochemical analysis indicated

lower concentrations of soluble Aβ peptides in double mutant mice, suggesting sequestration of toxic soluble Aβ oligomers into denser and less toxic aggregates.

Dubal et al. (2015) generated $APP_{swe/ind}$ (J20) mice that were also hemizygous for a Klotho transgene. $APP_{swe/ind}$ mice exhibit diminished brain Klotho levels compared to wild-type mice. Compared to $APP_{swe/ind}$ mice, double mutant mice exhibited reduced mortality, more normal EEGs with less epileptiform activity, and performed better on several behavioral assays. There was no effect of the Klotho transgene, however, on amyloid plaque burden, APP levels, Aβ peptide levels, APP degradation product levels, Tau levels, abnormally phosphorylated Tau levels, or neurite dystrophy. Klotho elevation did reduce hippocampal neuron spine loss, increased NMDA receptor subunit levels, and enhanced NMDA receptor dependent plasticity in the hippocampal formation.

In summary, these experiments describe positive effects of anti-aging interventions in murine genetic AD models. There are, however, significant disparities both within and between experiments. In some experiments, the IF study of Halagapa et al. (2007) and the Klotho cross experiment of Dubal et al. (2015), there was dissociation of behavioral effects and important pathologic endpoints. Taken together, these experiments do not reveal any consistent pattern of how anti-aging interventions affect pathogenesis in these models.

Huntington Disease

Three studies examined the effects of modulating aging in murine genetic models of HD (Table 2). Duan et al. (2003) applied IF in the N-171-82Q transgenic fragment model of HD (Schilling et al., 1999). In this line with an aggressive phenotype, IF was started at 8 weeks of age and increased survival, delayed the onset of motor dysfunction, and delayed the onset of weight loss, the latter despite IF. Histologic evaluation at 20 weeks of age demonstrated that CR reduced brain atrophy and reduced huntingtin protein aggregate deposition. N-171-82Q mice exhibited glucose intolerance that was improved significantly by IF, a potential confounding factor. IF also increased Brain Derived Neurotrophic Factor (BDNF) and HSP70 levels at 20 weeks of age.

Sadagurski et al. (2011) examined the effects of manipulating *IRS2* expression in another aggressive transgenic model of HD, R6/2 (Stack et al., 2005). Crossing R6/2 mice to mice harboring a neuron specific transgene ($IRS2^{ntg}$) resulted in an exacerbation of the R6/2 phenotype with accelerated weight loss, onset of motor dysfunction, and mortality. Sadagurski et al. (2011) also studied the effect of reducing IRS2 by crossing R6/2 mice to germline *IRS2* knockout mice also carrying a beta cell *IRS2* transgene (to prevent development of diabetes) to generate R6/2-$IRS2^{+/-}$-$IRS2_{\beta}^{tg}$ mice. Triple mutant mice exhibited amelioration of the R6/2 phenotype with delayed onset of motor dysfunction, delayed weight loss, and delayed mortality. At 11 weeks of age, R6/2-$IRS2^{+/-}$-$IRS2_{\beta}^{tg}$ mice exhibited less astrogliosis than R6/2 or $IRS2^{ntg}$ mice. At 6 and 11 weeks of age, R6/2-$IRS2^{+/-}$-$IRS2_{\beta}^{tg}$ mice exhibited reduced huntingtin protein inclusion burden compared to R6/2 or $IRS2^{ntg}$ mice, and there was less insoluble mutant huntingtin protein in the brains of 11 week old R6/2-$IRS2^{+/-}$-$IRS2_{\beta}^{tg}$ mice. Compared with R6/2 or $IRS2^{ntg}$ mice, R6/2-$IRS2^{+/-}$-$IRS2_{\beta}^{tg}$ mice exhibited biochemical markers of altered macroautophagy, increased

nuclear localization of the FoxO1 transcription factor, and increased expression of the FoxO1 regulated genes Peroxisome Proliferator-Activated Receptor gamma - Co-Activator 1 alpha and Superoxide Dismutase 2. Measures of oxidative stress and mitochondrial function were improved in R6/2-IRS2$^{+/-}$-IRS2$_{\beta}^{tg}$ mice relative to R6/2 or IRS2^{ntg} mice.

Tallaksen-Greene et al. (2014) used a more slowly progressive model of HD, heterozygous Q200 knockin mice, which exhibit behavioral and pathologic changes in the second year of life (Heng et al., 2010). These mice were crossed with the Snell dwarf (*Pitx1*$^{-/-}$), which exhibits marked retardation of aging (Flurkey et al., 2001). Multiple endpoints were examined with somewhat conflicting results. Compared with Q200 controls, Q200-*Pitx1*$^{-/-}$ mice exhibited reduced weight loss, and delayed onset and magnitude of motor dysfunction. Measures of pathology, including striatal huntingtin protein intranuclear inclusion burden and striatal dopamine receptor expression, were no different between Q200-*Pitx1*$^{-/-}$ and Q200 mice. Cytoplasmic huntingtin protein inclusions were probably reduced in striatal terminals but another marker of striatal terminal integrity was not different between between Q200-*Pitx1*$^{-/-}$ and Q200 mice.

As with experiments in AD models, positive effects of anti-aging interventions are reported. But, as found in AD model experiments, there are dissociations of behavioral and pathologic endpoints within some experiments (Tallaksen-Greene et al., 2014) and discrepant results between experiments.

Comment

While several of these experiments have produced findings suggesting that retarding aging may retard aspects of disease onset and/or progression in these models of neurodegeneration, the current data set has many gaps and weaknesses. Some of the genetic stocks that slow extended lifespan on standard backgrounds also delay behavioral changes or histopathologic abnormalities characteristic of these disease models, but there is inconsistency between behavioral and pathologic endpoints in most of the experiments. Interpretation of the published data is complicated by many factors, including the diversity of neurodegenerative models studied, and the diversity of anti-aging interventions tested, some of which have much larger, and more consistent, effects on lifespan than others.

In the AD model experiments, most studies report decreases in amyloid plaque burden or decreased accumulation of toxic Aβ peptides. Different experiments with different models and interventions, however, suggest very different mechanisms of action of anti-aging interventions. In the experiments of Halagappa et al. (2007) assessing CR and IF, these interventions produced similar behavioral effects but IF had no effect on accumulation of amyloidogenic Aβ peptides or abnormally phosphorylated Tau. Dubal et al. (2015) similarly reported significant behavioral effects of a Klotho transgene but no effects on APP levels, Aβ peptide levels, abnormally phosphorylated Tau protein, or histologic markers of neurodegeneration. Different experiments report different effects on APP and its toxic peptide products, some presenting evidence of altered APP processing, others suggesting increased Aβ peptide catabolism. The experiment of Cohen et al. (2009) suggested yet another mechanism, increased sequestration of toxic Aβ peptides by more densely packed

amyloid plaques. Some studies reported correlated effects on Aβ peptide levels and abnormally phosphorylated Tau, but Killick et al. (2009) found increased abnormally phosphorylated Tau accompanied decreases in Aβ peptide levels.

The smaller number of experiments using HD models appear to provide a more consistent picture, but one of these experiments, Tallaksen-Greene et al., (2014) report retardation of behavioral decline that does not seem to be attributable to parallel protection against histopathological effects of the HD gene.

An additional problem with interpretation of these experiments is uncertainty surrounding some of the anti-aging interventions. CR and the Snell dwarf mutation produce robust and well replicated effects on lifespan and many age-related physiological endpoints. Klotho transgenic mice are reported to exhibit substantial lifespan extension, but these results have not been replicated and effects of the Klotho transgene on age-associated pathologies have not been described (Kuroso et al., 2005). IF effects vary with mouse genotype and age of initiation of IF, with acceleration of mortality under some conditions (Fontana and Partridge 2015; Goodrick et al., 1990). While heterozygous IGF1R mice were reported originally to exhibit marked delay in aging, a subsequent replication experiment with more careful husbandry revealed only very modest effects in female mice (Holzenberger et al., 2003; Bokov et al., 2011). The status of IRS2 knockouts is controversial, and the data suggest that marked effects on aging may be present only in mice fed high fat diets (Taguchi et al., 2007; Selman et al., 2008; Selman et al., 2011). In the 2 AD experiments using IRS2 knockouts, conventional diets were used. The diet used in the HD model experiment of Sadagurski et al. is not reported.

It is also unclear whether the murine models of neurodegeneration employed in these experiments are appropriate surrogates for late-life human neurodegenerative illnesses. Most of the stocks used in this set of experiments are aggressive models in which significant pathology develops in the first year of life, often leading to death of the affected animals. Of the models studied, only the 3xTgAD mice used by Halagappa et al. (2007) and the Q200 HD mice used by Tallaksen-Greene et al. (2014) have slower onset phenotypes, though 3xTgAD mice express histopathologic changes in the first year of life. Whether aggressive models are really appropriate vehicles to examine anti-aging interventions is an important question. Among the HD lines, for example, R6/2 mice die by age 15–16 weeks and pathologic events begin in the first 3 weeks of postnatal life, analogous to the last human trimester of pregnancy (Stack et al., 2005). N-171-82Q mice nearly always die in the first few months of life (Schilling et al., 1999).

The R6/2 and N-171-82Q lines have an additional feature that may make them unsuitable for drawing inferences about the interaction of aging and Huntington disease. Both of these stocks exhibit glucose intolerance or frank diabetes (Duan et al., 2003; Hurlbert et al., 1999). Interventions like IF (Duan et al., 2003) or a beta cell IRS2 transgene (Sadagurski et al., 2011) might improve survival and phenotypic features by improving metabolic status independent of putative direct effects on neuronal function. In the CR-N-171-82Q experiment, IF normalized glucose metabolism. Improved glucose homeostasis might be particularly important in R6/2 mice. These mice frequently exhibit epilepsy and seizure

related mortality. Abnormal systemic metabolic factors, such as significant hyperglycemia, exacerbate seizure susceptibility. Recent data indicate that HD patients do not exhibit diabetes, glucose intolerance, abnormal insulin levels, or abnormal HOMA-IR (Wang et al., 2015; Boesgaard et al., 2009). Weight loss is a common problem in HD, with dysphagia and bradykinesia contributing to poor oral intake. Interventions such as CR or IF may be ill-advised in HD.

Another potential problem is inappropriate statistical design and inadequately powered experiments. It is not clear that statistical approaches that adequately account for interactions between interventions and disease states are always employed. Researchers should avoid the common error of inferring that a gene or drug or diet does not affect an endpoint of interest based on the observation that the effect size does not reach statistical significance in pair-wise or one-way comparisons. For designs in which the hypothesis of primary interest takes the form “Drug X slows the development of Disease X,” the analysis plan might require evaluation of the significance term of the interaction term of a two-factor ANOVA, in which the factors are “Drug X” and “Disease allele.” There are also problems inherent in using multiple readouts for intervention effects. These multiple comparisons are usually not corrected for Type I error and consequently underpowered. While evaluating many readouts is probably justified in exploring the potential mechanisms of interventions, evaluation of the efficacy of interventions requires carefully chosen and pre-specified endpoints using sufficient numbers of animals to provide adequate power (Landis et al., 2012; Llovera et al., 2015). Finally, greater attention probably needs to be paid to sex in design of these experiments. Some aging interventions have quantitatively different effects on males and females, and there may be sex differences in phenotypic severity in some neurodegeneration models. Both factors may have significant effects on experimental power, and it is not usually clear at the outset of an experiment whether the analysis must be done separately for male and female subjects.

While the concept that retardation of aging will have beneficial effects on neurodegenerative diseases has a good theoretical rationale and some experimental support, review of the published studies using murine genetic models reveals inconsistent results and several potential design flaws. Given the emergence of plausible drug candidates to retard aging, there is a real need for appropriately designed preclinical experiments exploring the possibility that drugs modulating aging will exert beneficial effects on neurodegenerative diseases. This may be especially pertinent for HD, where some data suggests that mTOR, a key node in pathways regulating aging, is a plausible therapeutic target (Lee et al., 2015). The prior experience reviewed here suggests several points to consider when designing these experiments (Table 3).

Experiments aimed at determining if interventions that slow aging retard neurodegenerative diseases in mice should use interventions with large, reproducible effects on lifespan, and, preferentially, established effects on other age-dependent physiological or pathological endpoints. To appropriately mimic the interactions of aging and neurodegeneration, use of aggressive neurodegeneration models with early onset phenotypes should be avoided in favor of models with phenotype onset resembling that of the corresponding human syndromes, e.g., models in which behavioral changes are noted in the last half of the normal

lifespan. As in well constructed clinical trials, there should be careful choice of endpoints and adequate statistical power. Studies assessing multiple endpoints, such as behavioral and pathologic outcome measures, should have explicit, pre-specified plans to deal with the possibility of conflicting endpoints. Statistical designs should account for interventions and disease states. Methods such as two factor ANOVAs that explicitly examine interactions between genotypes and interventions are often necessary. Sex effects of both interventions and phenotypic features of models should be considered in experimental design. Subject numbers may need adjustment to account for differences in sexes.

Acknowledgments

Supported by P30-AG024824 (RAM), R01-AG019899 (RAM), R56-NS082941 (RLA), and P50-NS091856 (RLA). The funding sources played no role in the preparation of or decision to submit this manuscript.

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Table 1

Study	Intervention	Neurodegeneration Model	Genetic Background	Endpoint s	Results	Endpoint Age(s)	Statistical Analysis
Patel et al., Neurobiol Aging 26:995–1000, 2005	Caloric Restriction (started at 14–15 weeks)	$APP_{swe/ind}$(J20) transgenic APP mice (K670N/M671L/V 717F mutations–PDGF promoter) Mucke et al., J Neurosci 20:4050–4059, 2000 APP+PS1 transgenic mice (PS1M146L–PDGF promoter) bred to Tg2576 APP mice – prion promoter Holcomb et al., Nat Med 4:97–100, 1998.	Not Stated Not Stated	Amyloid plaque number & size by IHC Astrocytosis by IHC Microglial Activation by IHC APP mRNA levels	APP+PS1 – decreased cortical, but not dentate or CA1 plaque area. $APP_{swe/ind}$ – decreased cortical and hippocampal plaque #; decreased plaque size. APP+PS1 – non significant effect on astrocytosis area; some effect close to astrocytes. Microglial effect inconclusive. No effect on APP mRNA	24 weeks	One-Way ANOVAs
Wang et al., FASE B J 19:659-651, 2005	Caloric Restriction (started at 3 months)	Tg2576 mice Hsiao et al., Science 274:99–102, 1996	C57B6/SJL	Aβ IHC & Stereology Aβ ELISA APP processing	Stable Weight, Decreased Fat Pad, Improved GTT Markedly decreased Aβ by IHC, ELISA Normal Total APP Possible increased α–secretase	12 Months	Two-Way ANOVAs
Halagappa et al., Neurobiol Aging 26:212-200, 2007	Caloric Restriction (started at 3 months) Intermittant Fasting (started at 3 months)	3xTgAD (APP_{swe}/Tau_{P301L}/ $PS1_{M146V}$) Oddo et al., Neuron 39:409–421, 2003	129/C57BL6	Open Field & Water Maze Aβ ELISA Tau Immunoblotting	CR: Improved Open Field & Water Maze; Reduced Aβ Peptides; Reduced pTau IF: Improved Open Field & Water Maze; No Change in Aβ Peptides or pTau	10 & 17 Months for Behavior 17 Months for Biochemistry	Two-Way ANOVAs & One-Way ANOVAs
Cohen et al., Cell 139:1157–1169, 2009.	IGF1-R Heterozygotes	APP_{swe}PS1ΔE9 mice Borchelt et al., Neuron 19:939–945, 1997	C3H/HeJ/C 57Bl6-J/129	Water Maze & Rotorod Astrocytosis IHC Neuronal Density & Synaptophysin IHC Aβ IHC & ELISAs EM In Vitro Aβ Aggregation Assay	Improved Water Maze & Rotorod Less Gliosis & Neuronal Loss More synaptophysin Plaque Burden Similar but Plaques Smaller & Denser More Aggregated Aβ & Less Soluble Abeta	11–15 months	Two-Way ANOVAs & One-Way ANOVAs
Freude et al., FASE B J	IRS2 KO IGF-1R(flox) IR(flox)	Hsiao et al., Science 274:99–102, 1996	C57BL/6	Mortality	IRS2KO Rescued Female but not Male	12 weeks & 48 Weeks	Kaplan-Meier & t-tests

Study	Intervention	Neurodegeneration Model	Genetic Background	Endpoint s	Results	Endpoint Age(s)	Statistical Analysis
23:3315–3324, 2009.	Synapsin Cre 5% Crude Fat Diet			APP ELISA & Immunoblotting	mortality; Some Effect on Euglycemic Males Reduced APP peptides; decreased CTFs (less processing) Effects of IGF-1R-Cre KO Similar (Hippocampus Only) IR and IGF1-R Heterozygotes Without Effects		
Killick et al., BBRC 386:257–262, 2009.	IRS2 KO 4% fat diet	Hsiao et al., Science 274:99–102, 1996	B6/SJL x C57BL/6	Tau Aβ IHC APP peptides Fear Conditioning	Increased Tau Phosphorylation Reduced Amyloid Plaque Burden No Change in APP Products Improved Fear Conditioning	12–15 months	One-Way ANOVAs & t-tests
Dubal et al., J Neurosci 35:2358–2371, 2015	Klotho Transgenic	J20 Mice	B6/C3H	Mortality Electrophysiology Water Maze & NOR APP & Tau Biochemistry NMDA Receptor Immunoblotting Neuronal Morphology	Reduced Mortality Less Seizure Activity Improved Water Maze & NOR No Change in APP or Tau Diminished Spine Loss Elevated NR1, NR2B, and Improved Synaptic Plasticity	3–12 Months	Log Rank, Mixed Model ANOVAs, Two Way ANOVAs, t-tests

PDGF – Platelet Derived Growth Factor; IHC – immunohistochemistry; pTau – abnormally phosphorylated Tau; GTT – glucose tolerance test; Aβ - amyloidogenic APP peptide fragments; CTFs – APP C-terminal fragments; IR – insulin receptor;IGF-1R – IGF1 receptor; NOR – novel object recognition test; NR1 – NMDA receptor subunit 1; NR2B – NMDA receptor subunit 2B.

Table 2

Study	Intervention	Neurodegeneration Model	Genetic Background	Endpoints	Results	Endpoint Age(s)	Statistical Analysis
Duan et al., PNAS 100:2911–2916, 2003	Intermittant Fasting	N-171-HD Schilling et al., Hum Mol Genet 8:397–407, 1999	C3H/HEJ x C57BL/6J	Mortality Rotorod Weight Loss Brain Atrophy & Inclusion Burden Caspase Activation Glucose Homeostasis BDBF & Chaperone Expression	Improved Survival Later Disease Onset Improved Rotorod Performance Delayed Weight Loss Reduced Brain Atrophy & Inclusion Burden Reduced Caspase Activation Better Glucose Control ImproveD BDNF, Chaperone Expression	20 weeks	ANOVAs (One-Way or Two-Way Not Specified); t-tests
Tallaksen-Greene et al., J Neurosci 34:15658–15668, 2014.	Snell Dwarf	Q200 Heng et al., Hum Mol Genet 19:3702–3720, 2010.	C57BL/6J x C3H/HeJ-DW/J	Body Weight Balance Beam Performance Striatal & Nigral Inclusion Burden Striatal & Dopamine Receptor Expression	Improved Body Weight Improved Balance Beam Performance Striatal Inclusion Burden Unchanged Nigral Inclusion Burden Improved No Change In Striatal or Nigral Dopamine Receptors	100 Weeks	Two-Way & One-Way ANOVAs
Sadagurski et al., JCI 121:4070–4081, 2011	IRS2 KO heterozygotes (spared Beta Cells) Diet not Described IRS2 Overexpression	R6/2 Stack et al., J Comp Neurol 490:354–370, 2005	C57BL/6-CBA x C57BL/6	Mortality Body Weight Rotorod Performance Inclusion Burden IRS2 Signaling Macroautophagy& FoxO1 Regulated Genes Oxidative Stress & Mitochondrial Function	Improved Mortality Improved Body Weight Slower Inclusion Accumulation Oxidative Stress Markers Diminished Improved Mitochondrial Function Increased Autophagosomes Increased Expression of FoxO1 Regulated Genes	6–20 Weeks	Kaplan-Meier; ANOVA s

Table 3

Recommendations for Future Experiments

- Experiments to see if interventions that slow aging also retard neurodegenerative diseases in mice should use interventions with large and reproducible effects on lifespan, and, preferentially, established effects on other age-dependent physiological or pathologic endpoints.
- To appropriately mimic the interactions of aging and neurodegenerative pathologies, use of aggressive neurodegeneration models with early onset phenotypes should be avoided in favor of models in which the timing of phenotype onset resembles more closely that of the corresponding human syndromes, e.g., in the last half of the normal lifespan.
- As in clinical trials, there should be careful choice of endpoints and studies should be appropriately powered. Experimental designs using more than one endpoint, e.g., both behavioral and pathologic outcome measures, should have explicit, pre-specified plans to deal with the possibility of conflicting outcomes.
- Appropriate statistical designs that account adequately for interactions between interventions and the disease states should be used. Methods such as two factor ANOVAs that explicitly examine interactions between interventions and genotypes are often necessary.
- Sex effects of both interventions and phenotypic features of models should be considered in designing experiments. Subject numbers may have to be modified to account for differences in sexes.