Aging is the primary risk factor for the majority of neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). There are almost 40 million people aged 65+ in the United States. Statistics predict that if you reach age 65 you can expect to live almost 20 more years. The average age of onset of PD is 60 years. If you are 85 years of age, you have an almost 50% risk of developing AD. The population of persons 85+ is projected to increase from 4.2 million in the year 2000 to 6.6 million in the year 2020. This suggests that these neurodegenerative diseases will reach a prevalence of epidemic proportions.

The current dogma holds that cellular mechanisms that are associated with aging and those that are related to neuron degeneration in PD and AD *are unrelated*. However, more recent evidence suggests that normal aging and the degeneration of specific neuron populations in AD or PD may be linked by the same cellular mechanisms. This remains a topic of debate. For your comprehensive exam, choose either AD or PD and use this disease to address the following questions:

1) Discuss what is known about one primary molecular mechanism that creates the pathology that occurs in one of these age-related neurodegenerative conditions. Discuss age-related changes in brain physiology that may promote, enhance, and/or allow the appearance of the disease specific pathology to be exaggerated in middle age and beyond.

2) For this section, assume that neurodegenerative disease exists along a continuum with natural aging. The implication of this is that if individuals survive long enough, it is inevitable that they will eventually develop AD or PD. Keep in mind that AD and PD are each viewed as genetically heterogeneous and complex disorders caused or influenced by multiple factors (e.g., specific genes, susceptibility alleles, environmental exposures, gene-environment interactions). Thus the development of "cause-directed therapies" is a goal of future research in these fields. Indeed, one model suggests that elements of lifestyle and genetics that promote healthy aging will decrease the incidence of these diseases in the general population. In this regard, discuss the following issues:

A.) how elements of lifestyle, genetics, or an intervention might impact the incidence of AD or PD.

B.) whether current data suggests a *single* target to cure AD or PD or if a multi-pronged approach is necessary.

C.) In the realm of "personalized medicine," how one could tailor a specific therapy or lifestyle for two patients with different "forms"* of AD or PD .

Introduction: Alzheimer's Disease

Alzheimer's disease (AD) currently affects approximately 36 million individuals worldwide, and is arguably one of the fastest growing neurological disorders. Considering that AD cost the world $604 billion in 2010, the growing burden of AD on the current and aging population and on our healthcare system is a major public health concern [1]. AD is classically characterized by enhanced cleavage of the amyloid precursor protein to yield the Aβ peptide that is capable of forming toxic amyloid plaques [1, 2]. AD is further characterized by cortical and hippocampal accumulation of intracellular neurofibrillary tangles (NFTs) composed of the microtubule associated protein tau [1]. Both Aβ plaques and NFTs can severely disrupt normal network activity and synaptic transmission ultimately resulting in neurodegeneration and cognitive decline. Normal physiological changes in the aging brain and those in AD are quite similar, and recent hypotheses postulate that AD-related dementia is almost inevitable beyond a certain age. That being said, both genetic and environmental factors influence the onset and severity of AD, and it is likely that recent advances in "personalized medicine" will be employed in treating individual cases of AD.

I. A Molecular Mechanism of AD Pathology: The Role of Apolipoprotein E4

Exorbitant research devoted to understanding the pathology underlying AD has primarily linked AD-related cognitive decline to extracellular accumulation of Aβ plaques and the intracellular development of NFTs. Increased Aβ deposition and reduced Aβ clearance may contribute to both reduced network activity and enhanced long-term depression (LTD), while NFT accumulation in dendritic spines may reduce spine growth and neurite extension [1, 3]. Genome-wide association studies have successfully identified a crucial molecule involved in both tau phosphorylation and Aβ -induced neurodegeneration, namely apolipoprotein E [4, 5]. Apolipoprotein E, specifically

Apolipoprotein E4 (apoE4), is a very-low-density lipoprotein primarily responsible for removing lipids from the circulation and transporting them to the liver [6, 7]. In the CNS, microglia and astrocytes produce apoE under normal physiological situations, while neurons rapidly induce apoE production under pathological conditions [8-10]. Due to its capacity to bind lipids and cholesterol, apoE expression by injured neurons is thought to be an attempt to scavenge lipids for membrane restoration [6, 7] . Yet neuronal production of apoE, particularly apoE4, can initiate severe toxic conditions within neurons, leading to a cascade of cellular mechanisms underlying AD pathogenesis.

Apolipoprotein E exists in three isoforms, E2, E3, and E4, that are directly encoded by the ε2, ε3, and ε4 alleles of the APOE locus on chromosome 19 [7, 11-17]. While the Apoε3/3 genotype appears to be the most common in humans, individuals possessing either one or two ε4 alleles are at a much greater risk for developing AD [17]. A total of 80% of individuals with early onset, familial AD and 65% of individuals with late onset, non-familial AD possess at least one ε4 allele, highlighting its potential role in the onset of AD pathology [17]. Moreover, individuals who possess two ε4 alleles have a 2.84-fold greater risk of developing AD, meaning they are eight times more likely to develop the disease [17]. Possessing the ε4 allele additionally lowers the age of AD onset, such that individuals with no ε4 alleles generally present with symptoms at 84 years of age, those with one ε4 allele present at 75 years of age, and those with two copies of ε4 exhibit AD symptoms at 64 years of age [17]. Recent genome-wide association studies consistently reveal that the most common genetic link between AD patients occurs at the APOE locus, where a single nucleotide polymorphism is significantly associated with the rate of cognitive decline [4, 5]. Notably, it is the distinct structural properties of the resulting apoE4 protein that potentiate its involvement in AD neurodegeneration and cognitive decline.

The critical feature of apoE4 that mediates its neurotoxicity is the presence of arginine residues at positions 112 and 158 of its primary amino acid structure [7, 11, 12, 16, 18-20]. The presence of Arg-112 allows apoE4 to undergo a phenomenon referred to as domain interaction, whereby Arg-61 of the C-terminal domain and Glu-255 of the N-terminal domain partake in a stable ionic bond [7, 11, 12, 16, 18-20]. The distinct Arg-112 residue of apoE4 allows the Arg-61 residue to extend away from the helical bundle of the protein and interact with the Glu-255 residue [18-20]. The ability for Arg-61 to extend away from the core protein structure is unavailable to the apoE2 and apoE3 isoforms, due to the presence of a cysteine residue at position 112 in both proteins [18-20]. In addition to the potential for domain interaction, the N-terminal denaturation curve of apoE4 involves the formation of a stable yet reactive folding intermediate, specifically referred to as a molten globule state [18, 21]. In the molten globule state, 1) a significant amount of the native protein's secondary structure is maintained, 2) the globule is structurally compact, and 3) the globule possesses enough mobility to expose its hydrophobic core [21]. The distinct ability for apoE4 to undergo domain interactions and to form a stable unfolding intermediate inherently render apoE4 more neurotoxic and more likely to induce neurodegeneration in ε4 allele carriers.

ApoE4 and Lysosomal Degradation in AD Pathology

The molten globule state of apoE4 potentiates Aβ-induced lysosomal degradation, a process that readily occurs in the case of Aβ accumulation in AD. Due to the exposure of its hydrophobic core, the molten globule intermediate of apoE4 can potently bind phospholipid membranes, alter membrane processes, and disrupt membrane integrity, particularly in lysosomes where content leakage can promote caspase-mediated cell apoptosis. A study by Ji et al., 2002 demonstrated that Neuro-2a cells transfected with apoE4 and treated with Aβ fragments exhibited drastic lysosomal

degradation and a high percentage of DNA fragmentation [22]. Furthermore, treatment with Z-VAD, an inhibitor of caspase activity, reduced the DNA fragmentation seen in apoE4/Aβ-treated cells, indicating that the DNA fragmentation and cell apoptosis occurred through caspase activation [22]. The particular folding pattern of ApoE4 thus allowed for enhanced lysosomal degradation, caspase activation, and subsequent neuronal apoptosis. Lysosomal interference is therefore a primary mechanism by which the apoE4 protein can rapidly induce neurodegeneration.

ApoE4 and Tau Phosphorylation in AD Pathology

In addition to its ability to react with phospholipid membranes and alter their normal function, the intermediate state of apoE4 exposes a key proteolytic cleavage site that results in the formation of a neurotoxic, C-terminal truncated form of the protein [13, 20]. While apoE3 readily binds to tau fibrils and reduces their susceptibility to hyperphosphorylation, the truncated form of apoE4 may potentiate tau phosphorylation (p-tau) and aggregate with p-tau into NFTs [8, 13, 23]. Using Neuro-2A cells transfected with either apoE3 or apoE4, Huang et al, 2001 showed that apoE4 cells transfected with the C-terminal truncated form of apoE4 selectively co-immunoprecipitated with p-tau and induced NFT-like structures within the cytoplasm [23]. Since both the truncated form of apoE4 as well as p-tau accumulate in the hippocampus of 18-21 month old (aged) mice, the age-dependent increase in both compounds selectively places ε4 carriers at a much greater risk for accelerated NFT formation [8]. Enhanced p-tau leads to an accumulation of toxic NFTs with a concomitant reduction in neurite extension, dendritic spine density, and dendritic arborization [3, 24, 25]. Thus, in addition to caspase activation and apoptosis, much of the accelerated cognitive decline seen in ε4 carriers may be a direct result of apoE4 fragments and their subsequent aggregation with p-tau.

ApoE4 and Disruption of GABAergic Transmission in AD Pathology

The toxic, C-terminal truncated fragments generated after proteolytic cleavage of the apoE4 protein selectively impair GABAergic interneuron function, particularly within the hippocampus of AD individuals. GABAergic transmission is impaired in AD individuals as shown by Seidl et al., 2001 who demonstrated a significant reduction in GABA in the temporal cortex, the occipital cortex, and the cerebellum of AD patients [26]. During normal learning, GABA release from hippocampual interneurons and inhibitory synaptogenesis on cortical dendritic spines facilitate efficient long-term potentiation (LTP) and memory production [13, 27, 28] . Additionally, newly-formed hippocampal neurons require GABAergic input for proper maturation and incorporation into existing circuits, suggesting that a reduction in GABAergic interneurons in AD patients impairs proper hippocampal neurogenesis [29]. In a recent study by Andrews-Zwilling et al., 2010, apoE4 knock-in mice experienced a significant reduction in the total number of GABAergic interneurons in the hilus of the hippocampus, with an associated reduction in total IPSP amplitude at GABAergic synapses [13]. Furthermore, apoE4 knock-in mice that performed most poorly in the Morris water maze task, a test of spatial learning and memory, had the lowest number of GABAergic interneurons in the hilus [13]. In the same study, 12-month old transgenic mice expressing the toxic apoE4 fragment but null for tau showed a significant restoration of GABAergic neurons in the hippocampus, indicating that the apoE4-induced degeneration is tau dependent [13]. Thus, apoE4 proteolytic cleavage yields toxic, cytoplasmic fragments of the protein that can escape the secretory pathway, phosphorylate tau proteins in the cytoplasm, impair hippocampal GABAergic transmission, and significantly impair learning and memory.

ApoE4 and Disruption of Reelin-Induced LTP in AD Pathology

A recently identified and intriguing relationship between apoE4 and synaptic plasticity involves the ability for the apoE receptor to induce NMDAR phosphorylation and potentiate LTP through its interaction with reelin. Reelin is a ligand primarily involved in neural migration during neural development [15]. In the adult brain, reelin acts to cluster apoE receptors at postsynaptic cell membranes to activate Src family non-receptor tyrosine kinases (SRK) [15, 30]. SRK's phosphorylate the modulatory subunits of N-methyl-D-aspartate receptors (NMDAR) enhancing their activity and facilitating LTP induction [15]. Binding of apoE4 to apoE receptors yields internalization of the ligand-receptor pair, after which the receptor is recycled to the membrane for use [31]. However, the folding pattern and domain interaction of apoE4 cause the endosome to potentially recognize the ligand-receptor pair as a foreign body worthy of degradation [16]. The apoeE4-apoE receptor pair remains within the endosome longer and receptor recycling is stalled [15, 31]. A study by Chen et al., 2010 showed that primary neurons specifically treated with apoE4 showed a significant reduction in membrane surface expression of the apoe2 receptor and a significant disruption of reelin-induced NMDAR activation and LTP [15]. A loss of LTP leads to dysfunctional synapses, dendritic collapse and a resulting neuronal loss, indicating that a loss of this interaction between apoE receptors and reelin can accelerate neuronal loss in AD patients carrying the ε4 allele [32].

II. Age-Related Changes in Brain Physiology and the Progression towards AD

Cellular and physiological changes associated with normal aging in the brain inevitably place the brain at a greater risk for neurodegeneration. In both aged and AD individuals, the pyramidal cells of the entorhinal cortex comprising the perforant pathway

as well as the CA1 subfield and the subiculum of the hippocampus are most vulnerable to the effects of both aging and AD [33]. Lost function or degeneration in the perforant pathway that connects the cortex to the hippocampus is primarily responsible for the mild or severe cognitive decline in aged or AD individuals, respectively [33]. Memory loss and cognitive decline associated with normal aging was initially thought to be the direct product of neuronal death in neocortical regions, CA1, and CA3 fields of the hippocampus, or layer II pyramidal cells of the entorhinal cortex connecting the neocortex to the hippocampus [33]. However, it is now known that one of the major distinctions between memory loss associated with normal aging and that associated with AD is that only AD patients exhibit significant neuronal death during the progression of the disease [33]. Three key components of the normal aging brain include NFT deposition in the superficial entorhinal cortex, altered calcium homeostasis, and dendritic spine loss, all of which contribute to the mild cognitive decline and memory impairment in aged individuals. Notably, severe alterations in any or all of these factors instantly places an individual at a much greater risk for developing the severe impairment and dementia associated with AD.

Aging and NFT Deposition

Layer II pyramidal cells of the entorhinal cortex in normal aged individuals are particularly susceptible to NFT formation, likely causing the usual memory loss seen in these individuals [33, 34]. Often, this memory decline is referred to as “benign senescent forgetfulness” in that it results from a mild disruption in the neocortical-hippocampal communication rather than the vast, NFT-induced degeneration of several cortical circuits in AD individuals [34]. Considering that both aged and AD brains contain NFT accumulation, the distinction between normal and pathologic NFT accumulation involves the location of NFT deposition [35, 36]. Though it has been reported that up to

90% of entorhinal pyramidal cells are lost in AD brains due to “end-stage” NFT accumulation, the composition and density of any given NFT is almost identical in aged and AD individuals [33, 34, 36]. Rather, the location of NFT deposition delineates an aged individual from a severely demented individual, with demented individuals showing NFTs in deeper, more widespread cortical areas [36].

One of the greatest predictors of whether this widespread NFT accumulation will occur is the APOE genotype of an individual. As discussed earlier, the ε4 allele puts individuals at a much greater risk for developing AD almost 10 years earlier than non-ε4 carriers [17]. The ε2/2 and ε2/3 genotypes are the least common in AD individuals, and ε2 carriers have a slower rate of hippocampal atrophy and significantly less CSF p-tau compared to ε3 carriers [37]. Furthermore, transgenic apoE3 mice are protected from NFT formation and the age-dependent increase in p-tau seen in apoE4 mice [8]. Enhanced p-tau is inherently linked to toxic apoE4 fragments within the neuronal cytoplasm, meaning ε4 carriers likely experience a much greater rate of NFT formation and deposition in higher cortical areas during the normal process of aging. While NFT deposition is a normal process in the aging brain, it can quickly become detrimental to cognitive health, in both ε4 and non-ε4 carriers alike, when it occurs in higher cortical structures and interferes with normal network activity.

Aging and the Dysregulation of Calcium Homeostasis

The first reports of altered calcium homeostasis in aged brains developed in the 1980’s in order to better understand the mechanisms behind the senescent physiology in the brain not explained by neuronal loss [38]. It is now widely accepted that altered calcium homeostasis within the aging brain is a major factor contributing to altered cell growth and death, altered synaptic plasticity, and an increase in long-term depression (LTD) [39, 40]. The major mechanism responsible for the alteration in calcium

homeostasis primarily occurs within the hippocampus and includes a loss of calcium influx through NMDARs, enhanced calcium influx through L-type voltage gated calcium channels, and enhanced calcium release from intracellular stores [39, 41]. In normal brain physiology, induction of LTP requires a rapid influx of calcium through NMDAR's, activation of calcium-dependent kinases, and subsequent activation of alpha-amino-3-hydroxy-5-methyl-4-isoxalone proprionate glutamate receptors (AMPAR) to facilitate the LTP [42]. Yet a modest rise in intracellular calcium, specifically through L-type calcium channels or from intracellular calcium stores, triggers dephosphorylation of AMPAR's and induction of LTD [41]. This causes a prolonged K+-induced after hyperpolarization (AHP) to control cell excitability, a key biomarker for the aged brain [40, 41]. Addtionally, In aged individuals the threshold for induction of LTD is greatly reduced while that for LTP is enhanced, making LTD more likely to occur and shifting the balance of LTD/LTP induction [41]. Thus, the AHP and the shift in the LTP/LTD balance mediated by an alteration in the calcium source for hippocampal neurons mediates the loss of synaptic transmission that presents as memory decline in aged individuals.

In addition to an imbalance in the cellular source of calcium, an imbalance in kinase and phosphatase activity, which is itself dependent on the source of intracellular calcium, also facilitates age-related decline in neuronal health. The aged brain is characterized by both a decrease in the basal level of kinase activity and a shift towards elevated phosphatase activity [39]. In particular, aged brains show an increase in the activity of calcineurin (CaN), a protein phosphatase activated by a modest rise in intracellular calcium specifically through L-type calcium channels [39, 41]. The rise in CaN potently affects both LTP, through activation of protein-phosphatase 1 (PP1), and general cell health, through inactivation of the cAMP response element binding protein (CREB) [39, 41]. PP1 activity reduces current through NMDARs and inactivates AMPAR's, both of which are responsible for initiating and maintaining LTP under normal

physiological conditions [39, 41]. On the other hand, inactivation of CREB can greatly reduce both memory and synaptic plasticity while enhancing cell susceptibility to neurotoxicity and apoptosis [39].

As in normal NFT deposition during aging, a drastic shift in the source of intracellular calcium during aging can quickly transition a relatively normal aging brain to one marked by pathological neurodegeneration. The increase in calcium influx through L-type channels and the increased release of calcium from intracellular stores both contribute to enhanced oxidative stress within neurons [39]. Under normal circumstances, circulating antioxidants are able to combat the detrimental effects of oxidative stress by scavenging free radicals, yet individuals with mild cognitive impairment (MCI) and AD are thought to be deficient in optimal antioxidant function [40]. While there is an attempt to initiate an antioxidant defense mechanism against oxidative stress in AD patients, the overall effect may not be efficient nor may it last long enough to remove reactive oxygen species. Mitochondrial dysfunction and altered glucose metabolism in individuals susceptible to AD, such as ε4 allele carriers, render vulnerable neurons in the hippocampus more likely to degenerate in response to enhanced oxidative stress after altered calcium homeostasis [43]. Moreover, enhanced calcium influx through L-type receptors activates CaN which can exacerbate cytokine release in the hippocampus through its interaction with nuclear factor of activated T-cells (NFAT) [44]. CaN facilitates the translocation of NFAT to the neuronal nucleus where it plays a role in activating the transcription of inflammatory cytokines [44]. This can lead to a destructive inflammatory cascade, reported to occur in AD, resulting in a rapid progression towards AD [44]. While the calcium-based physiological changes in the senescent brain are rather ubiquitous among all aging individuals, they initiate a series of cellular and physiological changes that can quickly tip the scale from normal aging to AD dementia.

Aging and Dendritic Spine Loss

An inevitable consequence of both altered calcium homeostasis and NFT deposition in superficial entorhinal cortex is a prominent loss of dendritic spine number and arborization in the aging brain. In studying the pyramidal neurons of both non-human primates and rats, researchers have found that the cortex is dominated by three major classes of dendritic spines, namely thin, mushroom, and stubby spines [45, 46]. Mushroom spines have large heads, a high concentration of AMPA receptors, and greater structural stability, due to their role in mediating memory storage and long-term memory formation [45, 46]. Thin spines which have few AMPA receptors are mostly dominated by NMDA receptors, and they function in new learning and new memory formation [45, 46]. By using Lucifer-Yellow to fill and identify spine type in aging cortical neurons, Dumitriu et al., 2010 showed that roughly half of the spine loss in aging in rhesus monkeys resulted from thin spine loss, with no significant loss of mushroom or stubby spines [47, 48]. Additionally, the size of the surviving thin spines in aged individuals increased significantly, and was tightly correlated to reduced performance on the delayed non-matching-to-sample task [48]. The authors suggested that the increased volume of thin spines with age compensated for the significant loss in total thin spine density, in an attempt to sustain cognitive functioning in the cortex [48].

The susceptibility of thin spines in cortical neurons to age-related loss explains much of the decline in cognitive tasks associated with aging, but hippocampal dendrites show a different pattern [45]. Larger, more complex synapses in the hippocampus appear to be more vulnerable to age-related decline and particularly to AD, ultimately leading to an impairment in hippocampal-dependent memory [45]. AD individuals show a particular defect in retaining new information when performing the delayed recognition span test, with a distinct impairment in both entorhinal and temporal cortices [49], Most

importantly, it is the cytoskeletal and neurophysiological alterations associated with normal aging that are primarily responsible for disrupting these higher order cortical and hippocampal circuits, ultimately leading to the cognitive and memory impairments associated with AD. Enhanced NFT deposition in cortical and hippocampal subfields drastically impairs re-growth of spines due to its effect on the neuronal cytoskeleton [3]. Additionally, enhanced CaN activity leads to enhanced NFAT translocation to the nucleus, where transcription and release of cytokines is detrimental to spine health [2]. It is likely that individuals who eventually develop AD have a drastic loss of thin and mushroom spine density within the deeper entorhinal cortex, CA1, and subiculum of the hippocampus leading to significant impairment in synaptic plasticity and LTP. Though spine loss in aging synapses is inevitable to the aging brain, exacerbation of the type and location of spine loss hastens the transition to AD and severe cognitive impairment.

III. The Impact of Lifestyle on the Incidence of AD: Obesity and Hyperinsulinemia

In light of the fact that there are currently 5.4 million cases of AD in the Unites States with 1 in every 3 U.S. adults being obese, it is necessary to investigate the link between obesity and the onset of AD in later life. Recent reports have provided a link between mid-life obesity and an increased risk for the eventual onset of AD [50]. It is likely that mid-life obesity alters several of the pathways involved in the pathogenesis of AD, and it confers an increased risk for AD even in the pre-clinical stages of the disease [50]. A meta-analysis by Profenno et al., 2009, reported that each 1 unit increase in BMI in 70, 75 or 79 year old women increased their risk for AD development between the ages of 79-88 [51]. Furthermore, when pooling data from obese and diabetic individuals and individuals reporting abnormal insulin or glucose, there was a substantial trend towards the development of AD [51]. Analyses by both Whitmer et al., 2007 and Fitzpatrick et al., 2009 reported that mid-life obesity rendered individuals more

susceptible to the later development of either dementia or AD [52, 53]. It is important to note that mid-life obesity rather than obesity later in life is distinctly associated with an increased risk for dementia. There is currently an ongoing “obesity paradox” whereby elderly individuals who are more overweight appear to be protected from more severe symptoms associated with dementia, due to the metabolic demands placed on the body during such a degenerative process [52]. Thus, it is crucial for young and middle aged individuals to promote healthy aging in an attempt to protect their brain from detrimental cognitive decline.

The link between mid-life obesity and an enhanced risk for AD may be the association between obesity and the occurrence of insulin resistance syndrome often prompted by obesity. In insulin resistance syndrome, insulin transport across the blood brain barrier is reduced, depriving the brain of normal insulin [54]. Insulin receptors are found in critical brain regions that are affected by AD, specifically the hippocampus, the entorhinal cortex and the frontal cortex, where insulin mediates the LTP required for memory consolidation [54]. Physiological concentrations of insulin are crucial to the normal function of the brain and to partially protecting the brain from NFT accumulation [55]. Insulin signaling activates phosphatidylinositol 3-kinase (PI3K) activity which inhibits glycogen synthase kinase-3 (GSK-3), a major kinase involved in tau phosphorylation [56]. There is, however, a fine balance between the concentration of insulin needed for normal brain functioning and protection, and the excessive concentration of peripheral insulin in individuals with hyperinsulinemia. Peripheral hyperinsulinemia is often associated with either a down-regulation of insulin receptors or a general dysfunction of these receptors, making the function of insulin negligible. In the CNS, hyperinsulinemia may either enhance Aβ plaque formation or reduce Aβ clearance, as noted by Watson et al., 2003 when insulin infusions into healthy adults resulted in a transient state of hyperinsulinemia and a concomitant increase in CSF Aβ

[57]. Additionally, insulin itself is degraded by the protease insulin degrading enzyme (IDE) the primary protease also responsible for Aβ plaque degradation [58]. Inhibition of IDE significantly reduces Aβ clearance within the brain, and a mutant allele in the IDE gene that renders the protease non-functional is linked to both hyperinsulinemia and reduced Abeta clearance [58]. It is therefore critical for middle-aged individuals to either avoid or reduce accumulation of the effects associated with obesity-induced hyperinsulinemia and/or insulin resistance. Insulin resistance and hyperinsulinemia deprive the brain of insulin either through disrupted BBB transport or reduced insulin receptor function, thereby potentiating both cognitive impairment and neurodegeneration. This intriguing link between obesity, hyperinsulinemia, and AD highlights how environment and lifestyle, even prior to the onset of dementia, play as crucial a role as genetic and cellular factors in the onset of AD.

IV. A Multi-Pronged Approach to Treating both Late and Early Onset AD

There exist two major forms of Alzheimer's disease, namely familial early onset and non-familial late onset, the latter of which represents a vast majority of AD cases. Considering that early onset or familial AD is directly linked to the genotype of the APOE gene, treatment regimens are much more specific, targeting the structural features of the apoE4 protein that are responsible for its neurotoxicity. Late onset cases, however, provide more of a challenge due to the multifactorial nature of late onset AD, involving both environmental and cellular changes in the aging brain. While both forms require a multi-pronged treatment regimen aimed at counteracting several of the degenerative processes in AD, more personalized therapeutics are implicated for either early or late onset individuals.

Treating Late Onset, Non-Familial AD

Current work aimed at targeting late onset AD has focused on potential mechanisms to reduce both amyloid plaque and NFT deposition, in hopes of restoring network connectivity and reducing synapse loss. Both NFTs and plaque deposition are implicated in late onset AD individuals meaning it is necessary to individually target each cellular pathology with distinct therapeutics. In order to target the amyloid plaques that plague AD brains, primary targets include either γ or β-secretase enzymes that are responsible for proteolytic cleavage of APP to yield Aβ peptide release [59]. Such attempts have, however, been primarily unsuccessful yielding unwanted side effects including an exacerbation of Aβ toxicity [59]. In order to enhance amyloid clearance, initial clinical trials attempting to induce an immune response against Aβ plaques were quickly arrested due to the occurrence of meningoencephalitis in 6% of the participants [60]. It is worthy to note, however, that 20% of the participants in the trial did experience the desired outcome of reduced Aβ plaque detriment, and future research in this field is warranted [60]. Fortunately, efforts aimed at targeting tau phosphorylation and NFT formation have been much more successful and may prove to be effective as a first line of defense against AD pathology. Roberson et al., 2007 reported that an *in vivo* reduction in total endogenous tau was capable of attenuating behavioral deficits in transgenic mice without any effect on Aβ plaque formation [61]. This suggests that tau contributes significantly to the cognitive decline in AD, independent of plaque formation, and that targeting endogenous tau has promising therapeutic potential. Much work remains to be done in order to safely and effectively target both plaque and NFT formation, though recent advances in understanding the mechanisms by which both Aβ and tau provoke neurodegeneration have provided the necessary initial steps.

Aside from the histopathological correlates of AD pathology, reactive oxygen species (ROS) inevitably increase in the aged brain, often to a point where significant

neural detriment can occur. Since AD individuals have a reduction in either the quantity or the quality of the antioxidant defense mechanism, antioxidant therapy may be a viable alternate approach for individuals initially presenting with mild AD symptoms. For example, mild cognitive impairment (MCI) has become the primary behavioral biomarker in predicting later progression to AD in elderly individuals [62]. It may be possible to employ an early intervention paradigm, particularly in individuals diagnosed with MCI, consisting of antioxidant treatment. Polyphenols have recently gained much attention as a complementary treatment for AD patients due to their neuroprotective effects. Polyphenols are natural metabolites with antioxidant properties found in natural fruits, vegetables, and herbs [63]. It appears as though a combination of early polyphenol or antioxidant treatment, in combination with later therapeutics targeting the histopathological changes associated with AD may be a future therapeutic option that could delay or reduce the onset of AD in future generations.

A final mechanism by which late onset, non-familial AD may be either delayed or prevented is by incorporating consistent cardiovascular exercise into one's daily regimen. Middle-aged individuals and aging individuals that may be on the verge of severe cognitive decline can combat much of the neurodegeneration associated with dementia through enhanced cardiovascular fitness (CFT). Not only is CFT the first line of defense against obesity, but it is also significantly linked to enhanced cognitive performance and overall brain health. CFT is associated with sustained cognitive function primarily through its ability to enhance brain-derived neurotrophin factor (BDNF) and other growth factors that promote neuronal survival and neurogenesis [64]. BDNF enhances synaptic development, a crucial process for maintenance of normal cognitive function and memory in the aging brain that is, as mentioned earlier, highly susceptible to dendritic spine and synapse loss [64]. Additionally, CFT has been linked to an enhancement of synapse formation, capillary formation, and connectivity within frontal

and parietal gray matter [64]. In a study by Colcombe et al., 2003, CFT significantly reduced the age-related decline seen in cortical areas most susceptible to synapse loss and dysfunction, namely the frontal, prefrontal, and parietal cortices [65]. Additionally, Colcombe et al., 2004 demonstrated that elderly participants undergoing a cardiovascular training regimen for 6 months showed a significant increase in task-related activity in such attentional control areas as the middle and superior frontal gyri [64]. Taken together, the benefits of CFT on the aging brain clearly promote a healthier brain able to combat oxidative stress and maintain cognitive function. Regular and optimal CFT is a significant lifestyle choice that should be incorporated as early as possible to both delay and reduce age-related neural dysfunction.

Treating Early Onset, Familial AD

In contrast to late onset non-familial AD, early onset or familial AD is linked to individuals with either one or two copies of the ε4 allele of the APOE gene. As discussed earlier, carriers of the ε4 allele are a significantly greater risk for developing AD at a much earlier age, primarily due to the domain interactions and the proteolytic cleavage that selectively occurs within the apoE4 protein. Though therapeutic options for early onset individuals should include all options discussed for late onset individuals, ε4 carriers present unique opportunities for therapeutic intervention. One such option is the use of small molecule correctors that would interfere with the domain interactions in the apoE4 protein [20]. Cultured Neuro2A cells and mouse primary hippocampal neurons expressing apoE4 were equally protected against much of the neural detriment associated with apoE4 after treatment with these small molecule correctors [16, 19]. Furthermore, a second possibility for ε4 carriers is inhibition of the protease responsible for cleavage of the apoE4 gene into the neurotoxic C-terminal truncated fragment. The specific protease responsible for apoE4 cleavage has been identified as a chymotrypsin-

like serine protease, and it is predicted that targeting this enzyme would be a highly efficient, personalized treatment for AD patients whose pathology is linked to the apoE4 protein [8]. The efficacy of personalized medicine is most notable in individuals possessing either one or two ε4 alleles at the APOE locus, and both preventative medicine (i.e. diet and exercise) and the aforementioned therapeutics may truly present an effective treatment plan.

Conclusion

The onset of Alzheimer's disease has risen dramatically within the past few decades, and the occurrence of the disease is projected to increase by a factor of three within the next 30 years. Cellular, environmental, and genetic factors play an equally important role in the onset of the disease, as do the normal physiological changes associated with the aging brain. In light of recent support for the efficacy of "personalized medicine", the future of Alzheimer's therapeutics will likely involve both general lifestyle modifications as well as specific therapies targeting the major players underlying Alzheimer's neurodegeneration.

References

1. Huang, Y. and L. Mucke, Alzheimer mechanisms and therapeutic strategies. Cell. 2012 Mar 16;148(6):1204-22.
2. Benilova, I., E. Karran, and B. De Strooper, The toxic Abeta oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci. 2012 Jan 29;15(3):349-57.
3. Nathan, B.P., et al., Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. Science. 1994 May 6;264(5160):850-2.
4. Bertram, L., C.M. Lill, and R.E. Tanzi, The genetics of Alzheimer disease: back to the future. Neuron. 2010 Oct 21;68(2):270-81.
5. De Jager, P.L., et al., A genome-wide scan for common variants affecting the rate of age-related cognitive decline. Neurobiology of Aging. 33(5): p. 15.
6. Mahley, R.W., Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science. 1988 Apr 29;240(4852):622-30.

7. Huang, Y., et al., Apolipoprotein E: diversity of cellular origins, structural and biophysical properties, and effects in Alzheimer's disease. J Mol Neurosci. 2004;23(3):189-204.
8. Brecht, W.J., et al., Neuron-specific apolipoprotein e4 proteolysis is associated with increased tau phosphorylation in brains of transgenic mice. J Neurosci. 2004 Mar 10;24(10):2527-34.
9. Harris, F.M., et al., Astroglial regulation of apolipoprotein E expression in neuronal cells. Implications for Alzheimer's disease. J Biol Chem. 2004 Jan 30;279(5):3862-8.
10. Buttini, M., et al., Cellular source of apolipoprotein E4 determines neuronal susceptibility to excitotoxic injury in transgenic mice. Am J Pathol. 2010 Aug;177(2):563-9.
11. Teter, B., et al., Defective neuronal sprouting by human apolipoprotein E4 is a gain-of-negative function. J Neurosci Res. 2002 May 1;68(3):331-6.
12. Huang, Y., Abeta-independent roles of apolipoprotein E4 in the pathogenesis of Alzheimer's disease. Trends Mol Med. 2010 Jun;16(6):287-94.
13. Andrews-Zwilling, Y., et al., Apolipoprotein E4 causes age- and Tau-dependent impairment of GABAergic interneurons, leading to learning and memory deficits in mice. J Neurosci. 2010 Oct 13;30(41):13707-17.
14. Filippini, N., et al., Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci U S A. 2009 Apr 28;106(17):7209-14.
15. Chen, Y., et al., ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. Proc Natl Acad Sci U S A. 2010 Jun 29;107(26):12011-6.
16. Brodbeck, J., et al., Structure-dependent impairment of intracellular apolipoprotein E4 trafficking and its detrimental effects are rescued by small-molecule structure correctors. J Biol Chem. 2011 May 13;286(19):17217-26.
17. Corder, E.H., et al., Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science. 1993 Aug 13;261(5123):921-3.
18. Zhong, N. and K.H. Weisgraber, Understanding the association of apolipoprotein E4 with Alzheimer disease: clues from its structure. J Biol Chem. 2009 Mar 6;284(10):6027-31.
19. Chen, H.K., et al., Small molecule structure correctors abolish detrimental effects of apolipoprotein E4 in cultured neurons. J Biol Chem. 2012 Feb 17;287(8):5253-66.
20. Mahley, R.W., K.H. Weisgraber, and Y. Huang, Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. Proc Natl Acad Sci U S A. 2006 Apr 11;103(15):5644-51.
21. Morrow, J.A., et al., Apolipoprotein E4 forms a molten globule. A potential basis for its association with disease. J Biol Chem. 2002 Dec 27;277(52):50380-5.
22. Ji, Z.S., et al., Apolipoprotein E4 potentiates amyloid beta peptide-induced lysosomal leakage and apoptosis in neuronal cells. J Biol Chem. 2002 Jun 14;277(24):21821-8.
23. Huang, Y., et al., Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons. Proc Natl Acad Sci U S A. 2001 Jul 17;98(15):8838-43.
24. Brodbeck, J., et al., Rosiglitazone increases dendritic spine density and rescues spine loss caused by apolipoprotein E4 in primary cortical neurons. Proc Natl Acad Sci U S A. 2008 Jan 29;105(4):1343-6.

25. Dumanis, S.B., et al., ApoE4 decreases spine density and dendritic complexity in cortical neurons in vivo. J Neurosci. 2009 Dec 2;29(48):15317-22.
26. Seidl, R., et al., Differences between GABA levels in Alzheimer's disease and Down syndrome with Alzheimer-like neuropathology. Naunyn Schmiedebergs Arch Pharmacol. 2001 Feb;363(2):139-45.
27. Cui, Y., et al., Neurofibromin regulation of ERK signaling modulates GABA release and learning. Cell. 2008 Oct 31;135(3):549-60.
28. Jasinska, M., et al., Rapid, learning-induced inhibitory synaptogenesis in murine barrel field. J Neurosci. 2010 Jan 20;30(3):1176-84.
29. Li, G., et al., GABAergic interneuron dysfunction impairs hippocampal neurogenesis in adult apolipoprotein E4 knockin mice. Cell Stem Cell. 2009 Dec 4;5(6):634-45.
30. Herz, J. and Y. Chen, Reelin, lipoprotein receptors and synaptic plasticity. Nat Rev Neurosci. 2006 Nov;7(11):850-9.
31. Heeren, J., et al., Impaired recycling of apolipoprotein E4 is associated with intracellular cholesterol accumulation. J Biol Chem. 2004 Dec 31;279(53):55483-92.
32. Palop, J.J., et al., Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron. 2007 Sep 6;55(5):697-711.
33. Morrison, J.H. and P.R. Hof, Life and death of neurons in the aging brain. Science. 1997 Oct 17;278(5337):412-9.
34. Vickers, J.C., A. Delacourte, and J.H. Morrison, Progressive transformation of the cytoskeleton associated with normal aging and Alzheimer's disease. Brain Res. 1992 Oct 30;594(2):273-8.
35. Abner, E.L., et al., "End-stage" neurofibrillary tangle pathology in preclinical Alzheimer's disease: fact or fiction? J Alzheimers Dis. 2011;25(3):445-53.
36. Braak, H., E. Braak, and J. Bohl, Staging of Alzheimer-related cortical destruction. Eur Neurol. 1993;33(6):403-8.
37. Chiang, G.C., et al., Hippocampal atrophy rates and CSF biomarkers in elderly APOE2 normal subjects. Neurology. 2010 Nov 30;75(22):1976-81.
38. Khachaturian, Z.S., Hypothesis on the regulation of cytosol calcium concentration and the aging brain. Neurobiol Aging. 1987 Jul-Aug;8(4):345-6.
39. Foster, T.C., Calcium homeostasis and modulation of synaptic plasticity in the aged brain. Aging Cell. 2007 Jun;6(3):319-25.
40. Foster, T.C., Biological markers of age-related memory deficits: treatment of senescent physiology. CNS Drugs. 2006;20(2):153-66.
41. Foster, T.C. and A. Kumar, Calcium dysregulation in the aging brain. Neuroscientist. 2002 Aug;8(4):297-301.
42. Kumar, A., K. Bodhinathan, and T.C. Foster, Susceptibility to Calcium Dysregulation during Brain Aging. Front Aging Neurosci. 2009 Nov 27;1:2.
43. Chen, H.K., et al., Apolipoprotein E4 domain interaction mediates detrimental effects on mitochondria and is a potential therapeutic target for Alzheimer disease. J Biol Chem. 2011 Feb 18;286(7):5215-21.
44. Abdul, H.M., et al., Cognitive decline in Alzheimer's disease is associated with selective changes in calcineurin/NFAT signaling. J Neurosci. 2009 Oct 14;29(41):12957-69.
45. Morrison, J.H. and M.G. Baxter, The ageing cortical synapse: hallmarks and implications for cognitive decline. Nat Rev Neurosci. 2012 Mar 7;13(4):240-50.
46. Kasai, H., et al., Structure-stability-function relationships of dendritic spines. Trends Neurosci. 2003 Jul;26(7):360-8.

47. Dumitriu, D., A. Rodriguez, and J.H. Morrison, High-throughput, detailed, cell-specific neuroanatomy of dendritic spines using microinjection and confocal microscopy. Nat Protoc. 2011 Aug 25;6(9):1391-411.
48. Dumitriu, D., et al., Selective changes in thin spine density and morphology in monkey prefrontal cortex correlate with aging-related cognitive impairment. J Neurosci. 2010 Jun 2;30(22):7507-15.
49. Albert, M.S., Cognitive and neurobiologic markers of early Alzheimer disease. Proc Natl Acad Sci U S A. 1996 Nov 26;93(24):13547-51.
50. Lee, E.B., Obesity, leptin, and Alzheimer's disease. Ann N Y Acad Sci. 2011 Dec;1243:15-29.
51. Profenno, L.A., A.P. Porsteinsson, and S.V. Faraone, Meta-analysis of Alzheimer's disease risk with obesity, diabetes, and related disorders. Biol Psychiatry. 2010 Mar 15;67(6):505-12.
52. Fitzpatrick, A.L., et al., Midlife and late-life obesity and the risk of dementia: cardiovascular health study. Arch Neurol. 2009 Mar;66(3):336-42.
53. Whitmer, R.A., et al., Body mass index in midlife and risk of Alzheimer disease and vascular dementia. Curr Alzheimer Res. 2007 Apr;4(2):103-9.
54. Craft, S., Insulin resistance syndrome and Alzheimer's disease: age- and obesity-related effects on memory, amyloid, and inflammation. Neurobiol Aging. 2005 Dec;26 Suppl 1:65-9.
55. Craft, S., et al., Insulin metabolism in Alzheimer's disease differs according to apolipoprotein E genotype and gender. Neuroendocrinology. 1999 Aug;70(2):146-52.
56. Hong, M. and V.M. Lee, Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons. J Biol Chem. 1997 Aug 1;272(31):19547-53.
57. Watson, G.S., et al., Insulin increases CSF Abeta42 levels in normal older adults. Neurology. 2003 Jun 24;60(12):1899-903.
58. Farris, W., et al., Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. Proc Natl Acad Sci U S A. 2003 Apr 1;100(7):4162-7.
59. Golde, T.E., L.S. Schneider, and E.H. Koo, Anti-abeta therapeutics in Alzheimer's disease: the need for a paradigm shift. Neuron. 2011 Jan 27;69(2):203-13.
60. Gilman, S., et al., Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology. 2005 May 10;64(9):1553-62.
61. Roberson, E.D., et al., Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. Science. 2007 May 4;316(5825):750-4.
62. Bozoki, A., et al., Mild cognitive impairments predict dementia in nondemented elderly patients with memory loss. Archives of Neurology, 2001. 58(3): p. 411-416.
63. Albarracin, S.L., et al., Effects of natural antioxidants in neurodegenerative disease. Nutr Neurosci. 2012 Jan;15(1):1-9.
64. Colcombe, S.J., et al., Cardiovascular fitness, cortical plasticity, and aging. Proc Natl Acad Sci U S A. 2004 Mar 2;101(9):3316-21.
65. Colcombe, S.J., et al., Aerobic fitness reduces brain tissue loss in aging humans. J Gerontol A Biol Sci Med Sci. 2003 Feb;58(2):176-80.
66. Mulkey, R.M., et al., Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature. 1994 Jun 9;369(6480):486-8.

67. Smith, D.L., et al., Reversal of long-term dendritic spine alterations in Alzheimer disease models. Proc Natl Acad Sci U S A. 2009 Sep 29;106(39):16877-82.
68. Tiraboschi, P., et al., Impact of APOE genotype on neuropathologic and neurochemical markers of Alzheimer disease. Neurology. 2004 Jun 8;62(11):1977-83.

Student 5:

Overall Analysis: Pass

The answer was provided an excellent overall analysis and discussion. It was well constructed, and a logical chain of events was outlined to implicate your selected molecular mechanism in causality. The document had good attention to details of mechanisms and excellent synthesis of the data presented. The answer demonstrates a good understanding of complex subjects and the student was able to extract pertinent details from the citations. While detail was extensive, it might have been a little too extensive in response to section 2 regarding contributing factors in brain aging. Lifestyle section was particularly well written as the student did a good job linking the incidence of AD to a potentially causal mechanism (hyperinsulinemia).

Overall evaluation: Strong overall.