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Allopregnanolone Levels are Reduced in Temporal Cortex in Patients with Alzheimer's Disease Compared to Cognitively Intact Control Subjects

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Abstract

Background—The neurosteroid allopregnanolone has pronounced neuroprotective actions, increases myelination, and enhances neurogenesis. Evidence suggests that allopregnanolone dysregulation may play a role in the pathophysiology of Alzheimer's disease (AD) and other neurodegenerative disorders. Our prior data demonstrate that allopregnanolone is reduced in prefrontal cortex in male patients with AD compared to male cognitively intact control subjects, and inversely correlated with neuropathological disease stage (Braak and Braak). We therefore determined if allopregnanolone levels are also reduced in AD patients compared to control subjects in temporal cortex, utilizing a larger set of samples from both male and female patients. In addition, we investigated if neurosteroids are altered in subjects who are APOE4 allele carriers.

Methods—Allopregnanolone, dehydroepiandrosterone (DHEA), and pregnenolone levels were determined in temporal cortex postmortem samples by gas chromatography/mass spectrometry, preceded by high performance liquid chromatography (40 subjects with AD/41 cognitively intact control subjects).

Results—Allopregnanolone levels are reduced in temporal cortex in patients with AD (median 2.68 ng/g, n= 40) compared to control subjects (median 5.64 ng/g, n=41), Mann-Whitney p=0.0002, and inversely correlated with Braak and Braak neuropathological disease stage (Spearman r= −0.38, p=0.0004). DHEA and pregnenolone are increased in patients with AD compared to control subjects. Patients carrying an APOE4 allele demonstrate reduced allopregnanolone levels in temporal cortex (Mann-Whitney p=0.04).

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Conclusions—Neurosteroids are altered in temporal cortex in patients with AD and related to neuropathological disease stage. The APOE4 allele is associated with reduced allopregnanolone levels. Neurosteroids may be relevant to the neurobiology and therapeutics of AD.

Introduction

Allopregnanolone is a neurosteroid with a number of properties that may be relevant to the pathophysiology and treatment of Alzheimer's disease (AD) and other neurodegenerative disorders, demonstrating pronounced neuroprotective actions in the setting of excitotoxicity [1,2], traumatic brain injury (TBI) [3–5], and neurodegeneration [6–8]. It also increases myelination [9–11], enhances neurogenesis [12], decreases inflammation [11,13,14], and reduces apoptosis [15–17]. Since excitotoxicity [18–21], neurodegeneration [22,23], and traumatic brain injury [24,25], as well as dysregulation in myelination [26,27], neurogenesis [28], apoptosis [29,30], and inflammation [31] have been implicated in the pathogenesis and clinical course of AD, deficits in allopregnanolone and/or alterations in its regulation could represent critical components of AD pathophysiology.

Emerging evidence demonstrating allopregnanolone deficits in neurodegenerative disorders is consistent with this hypothesis. For example, allopregnanolone levels are decreased in Niemann-Pick type C mice [7], a neurodegenerative disorder that shares a number of properties with AD. These include cholesterol dysregulation, neurofibrillary tangle formation, β-cleaved amyloid precursor protein accumulation, and myelin breakdown [28,32–37]. Further, allopregnanolone administration delays neurological symptom onset and doubles lifespan in Niemann-Pick type C mice [6–8]. Also consistent with a role for allopregnanolone in disorders in which neurodegeneration is a salient characteristic, allopregnanolone and other neurosteroids are altered in AD. Our laboratory determined previously that allopregnanolone levels in prefrontal cortex are significantly decreased in male AD patients compared to male cognitively intact control subjects, and that allopregnanolone levels are inversely correlated with neuropathological disease stage (Braak and Braak) [38]. Additional data also support the hypothesis that there may be allopregnanolone deficits in AD; for example, allopregnanolone is reduced in the periphery in serum [39] and plasma [40] in patients with AD compared to control subjects. Importantly, these earlier serum and plasma investigations also raise the possibility that other GABAergic neurosteroids (i.e. 3α-hydroxy-4-pregnen-20-one, [41,42]) with considerable cross-reactivity with the antibody used in radioimmunoassay procedures [40,43] may also be altered in AD.

It is possible that the determination of peripheral neurosteroid levels in blood may have proxy or surrogate biomarker potential for central neurosteroid levels in brain. Our prior efforts demonstrating that serum pregnenolone levels are closely correlated with hippocampal pregnenolone levels in rodents support this possibility [44]. Further, human data demonstrating that cerebrospinal fluid (CSF) levels of pregnenolone and dehydroepiandrosterone (DHEA) are correlated with temporal cortex levels of these respective neurosteroids within the same patient cohort are also consistent with proxy or surrogate biomarker potential for neurosteroid levels in more accessible tissues such as blood and CSF [45]. It should be noted, however, that several mechanisms for cerebral uptake of neurosteroids from peripheral blood circulation may influence central concentrations [46]. Given a compelling rationale informed by both preclinical and clinical findings from multiple research groups that implicate allopregnanolone dysregulation as a component in the pathophysiology of neurodegenerative disorders such as AD (and suggest a possible role for allopregnanolone or synthetic analogs in AD therapeutics), we thus investigated allopregnanolone levels in temporal cortex in patients with AD and cognitively intact control subjects. The overarching goal of the current study was to determine if we

could replicate our prior neurosteroid findings in prefrontal cortex in male AD and male control patients in a second brain region (temporal cortex) utilizing samples from a larger cohort of subjects that includes both male and female patients

In addition to allopregnanolone, other neurosteroids such as DHEA and pregnenolone may be candidate modulators of AD pathophysiology (see Figure 1 for biosynthetic pathways). For example, DHEA appears to be elevated in postmortem brain tissue [38,47] and CSF [45,47,48] in AD patients compared to control subjects, and positively correlated with Braak and Braak neuropathological disease stage [38]. Like allopregnanolone, DHEA demonstrates a number of neuroprotective effects. For example, DHEA is protective against amyloid β-protein toxicity [49,50] and a number of other insults involving oxidative stress, including anoxia [51], glucocorticoid-induced toxicity [52,53], and NMDA-induced excitotoxicity [54]. In addition, DHEA enhances neurogenesis in rodent models and augments cell proliferation of human neural stem cells [52,55,56]. Pregnenolone may also play a role in the pathogenesis and clinical course of AD, given its neuroprotective effects against glutamate [57] and amyloid β-protein toxicity [58], and actions on learning and memory in animal models [59,60]. We therefore determined DHEA and pregnenolone levels in this postmortem brain tissue investigation using temporal cortex samples from patients with AD and cognitively intact control subjects. Since the current study includes temporal cortex postmortem tissue from males and females, this larger collection of samples will also provide data to determine if our prior neurosteroid findings in male subjects are also generalizable to female patients. To our knowledge, this is among the largest postmortem brain tissue investigations focusing on neurosteroids and AD to date.

In addition, this larger cohort of 40 patients with AD and 41 cognitively intact control subjects provides the opportunity to conduct exploratory analyses to determine if the presence of the APOE4 allele (the ε4 allele of apoliprotein E, or ApoE), a known risk factor for the development of late onset AD [61], is associated with alterations in allopregnanolone, DHEA, and/or pregnenolone levels in temporal cortex. The mechanisms by which the APOE4 isoform of ApoE mediates AD risk are not yet completely understood. ApoE is a cholesterol transport protein that is present at high concentrations in the brain [62–64]. Since a major role of ApoE involves the regulation of cholesterol uptake into neurons (an action critical for synaptic function), and since cholesterol is the immediate precursor to pregnenolone (and pregnenolone is a precursor to other neurosteroids such as allopregnanolone), it is possible that AD risk conferred by the APOE4 allele may include a mechanism involving dysregulation in downstream events such as neurosteroid biosynthesis. To begin to test this possibility, we compared neurosteroid levels in temporal cortex in patients who are heterozygous or homozygous for the APOE4 allele, to subjects who do not carry this APOE isoform associated with elevated AD risk.

Methods and Materials

Postmortem Tissue

Frozen right hemisphere temporal cortex samples from a total of 81 patients were utilized in this investigation: samples from 40 subjects with AD (17 males, 23 females) and 41 cognitively intact control subjects (21 males, 20 females) from the Joseph and Kathleen Bryan Alzheimer's Disease Research Center (ADRC) collection at Duke University were analyzed for the neurosteroids allopregnanolone, DHEA, and pregnenolone by highly sensitive and specific gas chromatography/mass spectrometry (GC/MS) preceded by high performance liquid chromatography (HPLC) purification. A subset of this collection for which CSF was also available within the same cohort (n=41, approximately half of the total collection utilized for the current study) has been analyzed previously for pregnenolone and DHEA levels, and correlations of CSF pregnenolone and DHEA levels to respective

temporal cortex levels of these two neurosteroids have been reported in an earlier investigation [45]. Allopregnanolone levels in temporal cortex and APOE findings have not been reported previously (except in poster format, [65]). Temporal lobe boundaries were the superior and middle temporal gyri. Subjects were enrolled in the ADRC autopsy and brain donation program, as described previously [66]. Procedures for enrollment were approved by the Duke University Medical Center Institutional Review Board. Cognitively intact control subjects had no neurological disorders. AD was diagnosed clinically according to National Institute of Neurological and Communicative Disorders/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria. AD diagnosis was confirmed at autopsy using the National Institute on Aging/Reagan Institute criteria. Neuropathological disease stage was determined using the Braak method (Braak and Braak 1991). Postmortem interval (PMI) was less than 35 hours for all tissue samples tested.

Neurosteroid Analyses

Gas Chromatography/Mass Spectrometry Analyses (GC/MS) preceded by High Performance Liquid Chromatography (HPLC)—Neurosteroid analyses in temporal cortex tissue were performed as previously described [38,45,67], with minor modifications. All glassware was silanized. Between 100 and 180 mg of brain tissue was homogenized in 5 volumes of distilled water containing trace quantity (4000 dpm/injection) of tritiated neurosteroid (New England Nuclear Life, Wellesley, MA) to detect the HPLC fraction containing the neurosteroid of interest, and a constant amount of deuterated allopregnanolone (D4-allopregnanolone, 400 pg) and deuterated pregnenolone (D4-pregnenolone, 400 pg) as the internal standards (Cambridge Isotopes, Andover MA). Supernatants were extracted three times with three volumes of ethyl acetate and dried under nitrogen prior to HPLC purification, performed with 900 μL injections per sample on an 1100 Series Agilent HPLC equipped with a Packard 500TR Flow Scintillation Analyzer for radiopeak detection. Each neurosteroid was collected into a separate fraction based upon the retention time of its radioactive analogue, utilizing hexane, tetrahydrofuran, and ethanol as the mobile phase and a Phenomenex LiChrosorb DIOL (5 μm particle size) 250 mm × 4.6 mm column. The HPLC fractions containing allopregnanolone, DHEA, and pregnenolone were transferred to 1 mL Reacti-Vials, evaporated to dryness, and derivatized utilizing heptafluorobutyric acid anhydride (HFBA) [50 μL HFBA added to 450 μL ethyl acetate at room temperature for 2 hours]. Derivatized samples were transferred to autosampler vials equipped with deactivated glass inserts using 2 × 50 μL pesticide-grade heptane and evaporated, then reconstituted and vortexed in pesticide-grade heptane. Standards and samples were injected onto an Agilent 5973 Mass Spectrometer (MS) coupled to an Agilent 6890N Gas Chromatograph (GC) equipped with an Agilent HP-5MS 30 meter × 0.250 mm × 0.25 um capillary column, and analyzed in the negative ion chemical ionization mode (NICI) utilizing methane as the reaction gas and helium as the carrier gas. The derivatized steroids of interest subjected to NICI yield negative ions in the mass range between m/z 100 and m/z 700. In addition to the GC retention time characteristic of each steroid, the structural identification of each neurosteroid assayed was provided by its unique mass fragmentation pattern. Mass spectrometer single ion monitoring mode was utilized to focus on the most abundant ion fragment for each steroid derivative (allopregnanolone = 474.4 and 494.3; DHEA = 464.4 and 444.4; pregnenolone = 492.3 and 472.4).

For neurosteroid quantification, the standard curve for the steroid of interest was prepared by combining varying known quantities of the steroid (Steraloids, Newport, RI) with a constant amount of deuterated internal standard. Identical to the samples, the standard curve was extracted three times in ethyl acetate prior to HPLC purification and GC/MS injection (standard curve r^2=0.99 for each neurosteroid). The area under the peak of each known quantity of neurosteroid was divided by the area under the peak of the internal standard.

This ratio was then plotted on the y-axis against known quantities of each steroid to generate the standard curve. Only peaks with a signal to noise ratio greater or equal to 5:1 were integrated. The limit of detection with this method was 2 pg for allopregnanolone, 2 pg for DHEA, and 5 pg for pregnenolone.

Statistical Analysis

Non-parametric statistical approaches were utilized. Neurosteroid levels in AD patients and cognitively intact control subjects were analyzed by Mann-Whitney *U* test statistic. Correlational analyses (neurosteroid levels vs. PMI, and neurosteroid levels vs. Braak and Braak staging) were also assessed non-parametrically and Spearman correlation coefficients were determined. Both AD subjects and cognitively intact control subjects were included in the correlational analyses of neurosteroid levels vs. Braak and Braak neuropathological disease stage, since cognitively intact control subjects without clinical evidence of AD may meet neuropathological criteria for early Braak stages, and since these changes may reflect early stages of AD (or predisposition to developing AD) in the absence of detectable clinical symptomatology. P values < 0.05 were considered to be statistically significant.

Results

Median PMI was 6.33 hours for the Alzheimer's group and 7.68 hours for the cognitively intact control group (See Table 1 for patient characteristics). There was no significant difference in median PMI in the Alzheimer's group compared to the cognitively intact control group (Mann-Whitney *U* test statistic p=0.20). Median age was 81.0 years for the Alzheimer's group and 82.0 years for the cognitively intact control group. There was no significant difference in median age in the Alzheimer's group (n=40) compared to the cognitively intact control group (n=41), Mann-Whitney *U* test statistic p=0.44. None of the three neurosteroids tested (allopregnanolone, DHEA, pregnenolone) demonstrated significant correlations with PMI (Spearman $p \geq 0.40$ for each neurosteroid).

Allopregnanolone Levels in Temporal Cortex in Patients with AD vs. Control Subjects, and Relationship to Neuropathological Disease Stage (Braak and Braak)

Allopregnanolone levels are significantly decreased in temporal cortex in patients with AD (median 2.68 ng/g, n=40) compared to cognitively intact control subjects (median 5.64 ng/g, n=41), Mann-Whitney p=0.0002 (Figure 2, Table 2). These findings are very similar to those previously reported in prefrontal cortex, in which median allopregnanolone level in male patients with AD was 2.50 ng/g (n=14), and median allopregnanolone level in male cognitively intact control subjects was 5.59 ng/g (n=15), using the same mass spectrometry-based methodology [38]. Also consistent with our prior investigation in prefrontal cortex, allopregnanolone levels are inversely correlated with Braak and Braak neuropathological disease stage in the current study, Spearman $r=-0.38$, p=0.0004 (Figure 3), thus replicating our previous findings in prefrontal cortex in which an inverse correlation with Braak and Braak stage was also reported (Spearman $r=-0.49$, p=0.007) [38].

DHEA and Pregnenolone Levels in Temporal Cortex in Patients with AD vs. Control Subjects, and Relationship to Neuropathological Disease Stage (Braak and Braak)

DHEA levels are significantly elevated in temporal cortex in the current study in subjects with AD (median DHEA level 4.75 ng/g, n=40) compared to cognitively intact control subjects (median DHEA level 2.23 ng/g, n=41), Mann-Whitney p=0.0185 (Figure 4, Table 2). Pregnenolone levels are also significantly elevated in patients with AD (median pregnenolone level 22.24 ng/g, n=40) compared to cognitively intact control subjects (median pregnenolone level 10.24 ng/g, n=41), Mann-Whitney p=0.022 (Figure 5, Table 2). Both DHEA levels in temporal cortex (Spearman r= 0.26, p=0.017, n=81; Figure 6) and

pregnenolone levels in temporal cortex (Spearman r=0.24, p = 0.032, n=81; Figure 7), are positively correlated with Braak and Braak neuropathological disease stage.

DHEA levels are significantly higher in female patients compared to male patients, in both the AD group (6.48 vs. 2.85 ng/g, respectively; p=0.04) and the cognitively intact control group (3.39 vs. 2.04 ng/g, respectively; p=0.036). There are no significant differences in pregnenolone or allopregnanolone levels in female patients compared to male patients (in either the AD group or the cognitively intact control group), data not shown.

APOE4 Allele Status and Neurosteroid Levels

APOE status was available for 80 of 81 subjects. Allopregnanolone median level in temporal cortex is significantly decreased in patients homozygous or heterozygous for the APOE4 allele (2.86 ng/g, n=36) compared to patients not carrying an APOE4 allele (5.23 ng/g, n=44), Mann Whitney p=0.04; Figure 8. Patients homozygous or heterozygous for the APOE4 allele did not demonstrate significantly different median DHEA or pregnenolone levels compared to subjects who are not APOE4 allele carriers (data not shown).

Discussion

This investigation demonstrates that neurosteroid levels are altered in temporal cortex in patients with AD compared to cognitively intact control subjects using a postmortem brain tissue collection that includes both male and female patient samples (n=81; 40 AD, 41 control). Findings are consistent with our prior study in prefrontal cortex, using a smaller collection of samples from male patients only (n=29; 14 AD, 15 control) [38]. In the current study in temporal cortex, allopregnanolone levels are significantly reduced in AD patients compared to cognitively intact control subjects, and inversely correlated with Braak and Braak neuropathological disease stage (replicating our prior findings in prefrontal cortex). DHEA and pregnenolone levels, conversely, are significantly elevated in patients with AD compared to cognitively intact control subjects, and positively correlated with Braak and Braak neuropathological disease stage (also similar to our earlier findings in prefrontal cortex). In addition, the presence of the APOE4 allele is associated with neurosteroid alterations; specifically, subjects either heterozygous or homozygous for the APOE4 allele demonstrate significantly reduced allopregnanolone levels compared to patients who do not carry this APOE isoform. These findings and their potential ramifications are discussed below.

Allopregnanolone Levels in Temporal Cortex Are Reduced in Alzheimer's Disease and Inversely Correlated with Neuropathological Disease Stage

Allopregnanolone levels in temporal cortex are significantly decreased in patients with AD, similar to our prior findings in a smaller study in prefrontal cortex that included samples from males only [38]. Allopregnanolone levels are also inversely correlated with neuropathological disease stage (Braak and Braak), suggesting that decreases in this neurosteroid may have functional relevance and reducing the likelihood that the finding of decreased allopregnanolone levels in AD is simply an epiphenomenon. Replication of our prior prefrontal cortex allopregnanolone results in a second brain region (and extension of earlier prefrontal cortex findings to female subjects) further strengthens the rationale for the preliminary hypothesis that a dysequilibrium in neurosteroid pathways may contribute to the pathogenesis of AD. As previously noted, allopregnanolone is a molecule with pleiotropic actions, including pronounced neuroprotective effects against several processes associated with AD risk and/or pathophysiology; deficits in this neurosteroid may thus impact multiple dimensions of AD neurobiology and merit additional investigation.

In addition to the possible role of allopregnanolone reductions in AD pathogenesis, converging preclinical and clinical evidence also support the preliminary hypothesis that restoration of allopregnanolone levels in states of deficiency could be clinically therapeutic. For example, allopregnanolone is significantly decreased in Niemann-Pick type C mice, and one-time administration of this neurosteroid doubles lifespan and markedly delays neurological symptom onset [6–8]. The mechanisms by which allopregnanolone exerts these robust effects may include $GABA_A$ receptor modulation and activity at pregnane-X-receptors [8]. Further, allopregnanolone administration is neuroprotective against TBI [3,4,12,13] and stroke [68] in rodent models, to an even greater degree than its precursor, progesterone. Clinically, progesterone reduces death by 50% at 30 days post-injury in patients with moderate to severe TBI [69], a finding that has been replicated in an independent cohort [70] and led to a multi-site randomized controlled trial projected to enroll over 1100 patients (ClinicalTrials.gov Identifier: NCT00822900). Given the superior neuroprotective effects of allopregnanolone compared to progesterone in TBI and stroke rodent models, it is possible that progesterone metabolism to allopregnanolone may represent a mechanistic component to its therapeutic efficacy. This is further supported by prior work demonstrating that the anticonvulsant effects of progesterone are prevented if metabolism to allopregnanolone is blocked with an enzyme inhibitor [71–73]. Data are hence accruing to suggest that allopregnanolone may be a key modulator of neurobiological events in disorders in which neurodegeneration is a prominent component.

DHEA Levels in Temporal Cortex are Elevated in Alzheimer's Disease and Positively Correlated with Neuropathological Disease Stage

Our current findings suggest that temporal cortex DHEA levels are significantly increased in patients with AD compared to cognitively intact control subjects, and that DHEA levels in this brain region are positively correlated with Braak and Braak neuropathological disease stage (replicating our prior findings in prefrontal cortex [38], and extending this earlier effort by including samples from female subjects). Elevation in median DHEA level in temporal cortex in this investigation is also consistent with prior evidence of DHEA increases in CSF in subjects with AD [45]. As DHEA levels decline precipitously with age to levels approximately 20% of those observed in young adulthood, it has been hypothesized that decreases in DHEA may be linked to AD pathophysiology. Along these lines, evidence that DHEA protects against amyloid β-protein toxicity [49], attenuates $\beta_{25–35}$-amyloid peptide-induced memory impairment [74], and inhibits amyloid β-protein-induced calcium increases [75], supports the possibility that DHEA elevations in temporal cortex in AD patients could be compensatory in some manner. Also, the administration of β-amyloid peptide produces elevations in DHEA [47], and it is thus possible that β-amyloid deposition precedes DHEA alterations in AD and potentially plays an etiological role in the DHEA changes observed in temporal cortex in the current study. DHEA enhancement of axonal outgrowth [76] and other neurotrophic and neuroprotective actions previously outlined in the introduction are also consistent with this possibility, as are the positive effects of DHEA on learning and memory in rodent models [59,60,77]. Alternatively, DHEA positively modulates excitatory NMDA receptors [78,79] and negatively modulates inhibitory $GABA_A$ receptors [80]; therefore, this neurosteroid could potentially contribute to excitotoxic mechanisms in AD (particularly in the setting of concurrent deficits in allopregnanolone, an inhibitory GABAergic neurosteroid).

Pregnenolone Levels in Temporal Cortex are Elevated in Alzheimer's Disease and Positively Correlated with Neuropathological Disease Stage

Similar to the DHEA findings described above, pregnenolone levels in temporal cortex are also significantly elevated in patients with AD, and positively correlated with Braak and Braak neuropathological disease stage. Like allopregnanolone and DHEA, pregnenolone

demonstrates a number of properties that suggest it may be a logical candidate for future study in AD, such as enhancing learning and memory and increasing long-term potentiation in rodents [59] [81]. Given its diverse effects including microtubule stabilization [82], positive actions on neuritic outgrowth [83], the enhancement of myelination [84], protection against glutamate-induced cellular damage [57], prevention of increases in intracellular calcium induced by β-amyloid protein [75], and protection of mouse hippocampal (HT-22) cells against β-amyloid protein toxicity [58], pregnenolone elevations in AD could result in pleiotropic actions that impact a number of neurobiological processes relevant to AD. However, the precise etiology of these alterations and their functional consequences will require extensive additional study.

APOE4 Allele Status and Neurosteroid Levels in Temporal Cortex

Patients either homozygous or heterozygous for the APOE4 allele also had significantly reduced allopregnanolone levels compared to subjects without this APOE isoform. Since ApoE is a major cholesterol transport protein in the brain, and since cholesterol is the immediate precursor to pregnenolone (and pregnenolone is a precursor for multiple other downstream neurosteroids, including allopregnanolone and DHEA), it is possible that the APOE4 allele may be associated with compromised neurosteroid regulation by impacting upstream events involving cholesterol regulation and metabolism. This may be relevant to pregnenolone alterations in AD, since the conversion of cholesterol to pregnenolone is the rate-limiting step in neurosteroid formation [85,86]. Recent evidence that mRNA levels of diazepam binding inhibitor (which facilitates cholesterol transport to the inner mitochondrial membrane, an action required for pregnenolone synthesis) are elevated in patients with AD [87] is potentially consistent with our finding of elevated pregnenolone levels in temporal cortex in AD patients compared to cognitively intact control subjects. Conversely, however, the presence of the APOE4 allele is not associated with alterations in pregnenolone (or DHEA) in temporal cortex in this cohort, unlike the association of the APOE4 allele with significantly reduced allopregnanolone levels in this study. Of note, it is possible that the neurosteroid allopregnanolone may also play a role in cholesterol homeostasis, as allopregnanolone administration at postnatal day 7 significantly reduces neuronal and microglial cholesterol accumulation in Niemann-Pick type C mice [8,11]. Precisely how the presence of the APOE4 allele may influence neurosteroid regulation will require future investigation, but initial evidence that the presence of this isoform is associated with significant reductions in allopregnanolone merit additional study. Since accruing data strongly suggest that alterations in cholesterol metabolism are involved in the pathogenesis of AD [64,88], the investigation of downstream neurosteroid biosynthesis represents a logical avenue for future examination.

Study Limitations

Limitations of this investigation in temporal cortex postmortem tissue include a relatively small sample size (40 subjects with AD and 41 cognitively intact control subjects). However, the current investigation is more than twice as large as our prior study in prefrontal cortex [38], and also larger than a number of published postmortem brain tissue investigations focusing on neurosteroids and AD [67,89,90]. Another limitation that is frequently unavoidable in postmortem brain tissue studies is inability to control for medication status at the time of death, for either AD patients or cognitively intact control subjects. Since some pharmacological agents increase allopregnanolone [51,91–97] and pregnenolone [44,95,98], this could be a confounding element. In addition, smoking status at the time of death is not known for this cohort, and smoking is associated with elevations in DHEA [99] and may also be associated with elevations in allopregnanolone and pregnenolone [100].

Summary

In congregate, the results of this investigation are consistent with a role for neurosteroids in the pathophysiology and therapeutics of AD. In light of recent converging preclinical and clinical evidence linking allopregnanolone deficits to neurodegenerative disorders, restoration of this neurosteroid may represent a logical treatment strategy. Since allopregnanolone levels are inversely related to Braak and Braak neuropathological disease stage, reductions in this neurosteroid may have functional significance and relevance to illness progression. Decreases in allopregnanolone levels in the presence of the APOE4 allele, a well-established risk factor for late-onset AD, introduce additional lines of investigation for future exploration and hypothesis-testing. Findings that DHEA and pregnenolone are elevated in AD compared to cognitively intact control subjects (and positively correlated with Braak and Braak neuropathological disease stage) suggest altered regulation of neurosteroid biosynthetic pathways, potentially involving the accumulation of pregnenolone precursor and blockage in downstream allopregnanolone formation. Additional efforts and replication of these findings will be required to examine these possibilities.

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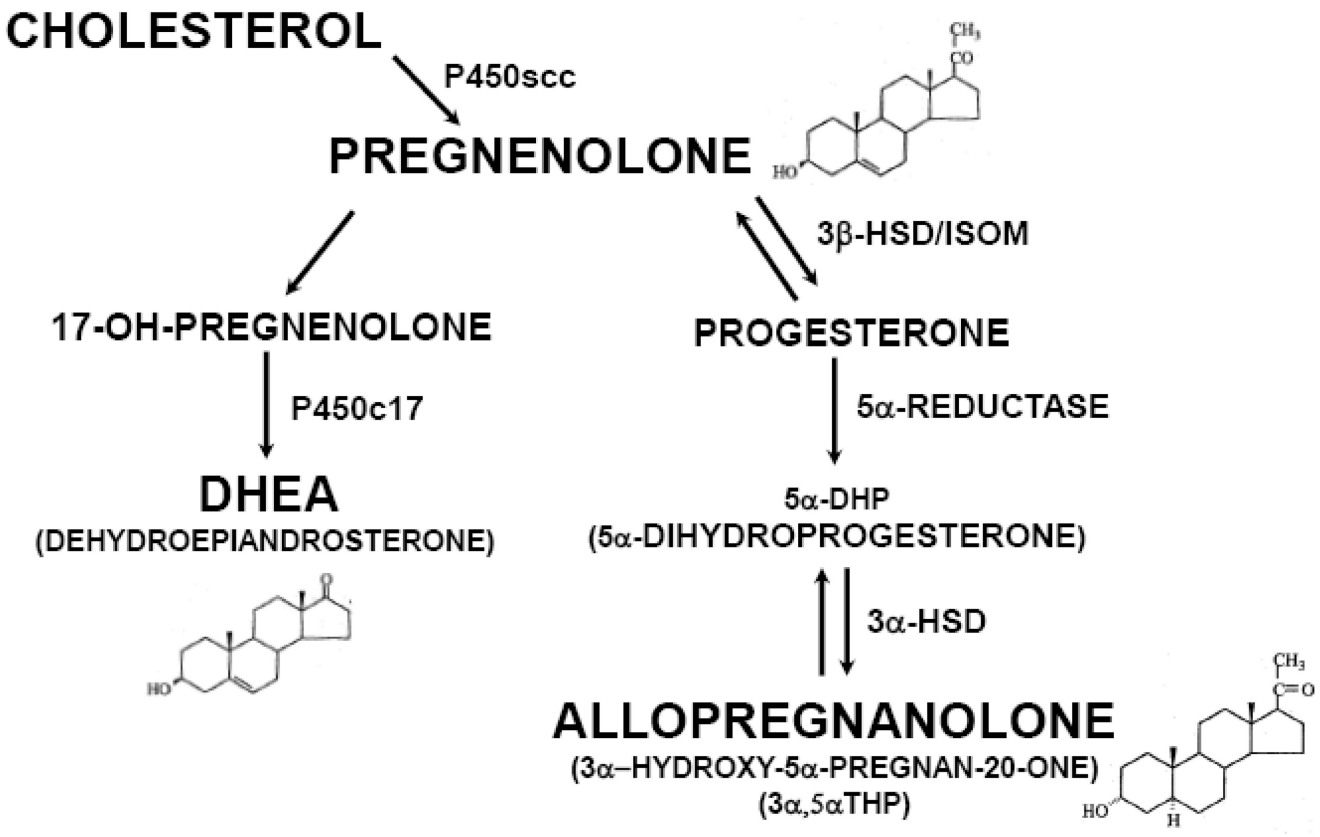

Figure 1.
Biosynthetic Pathways and Chemical Structures for Selected Neurosteroids.

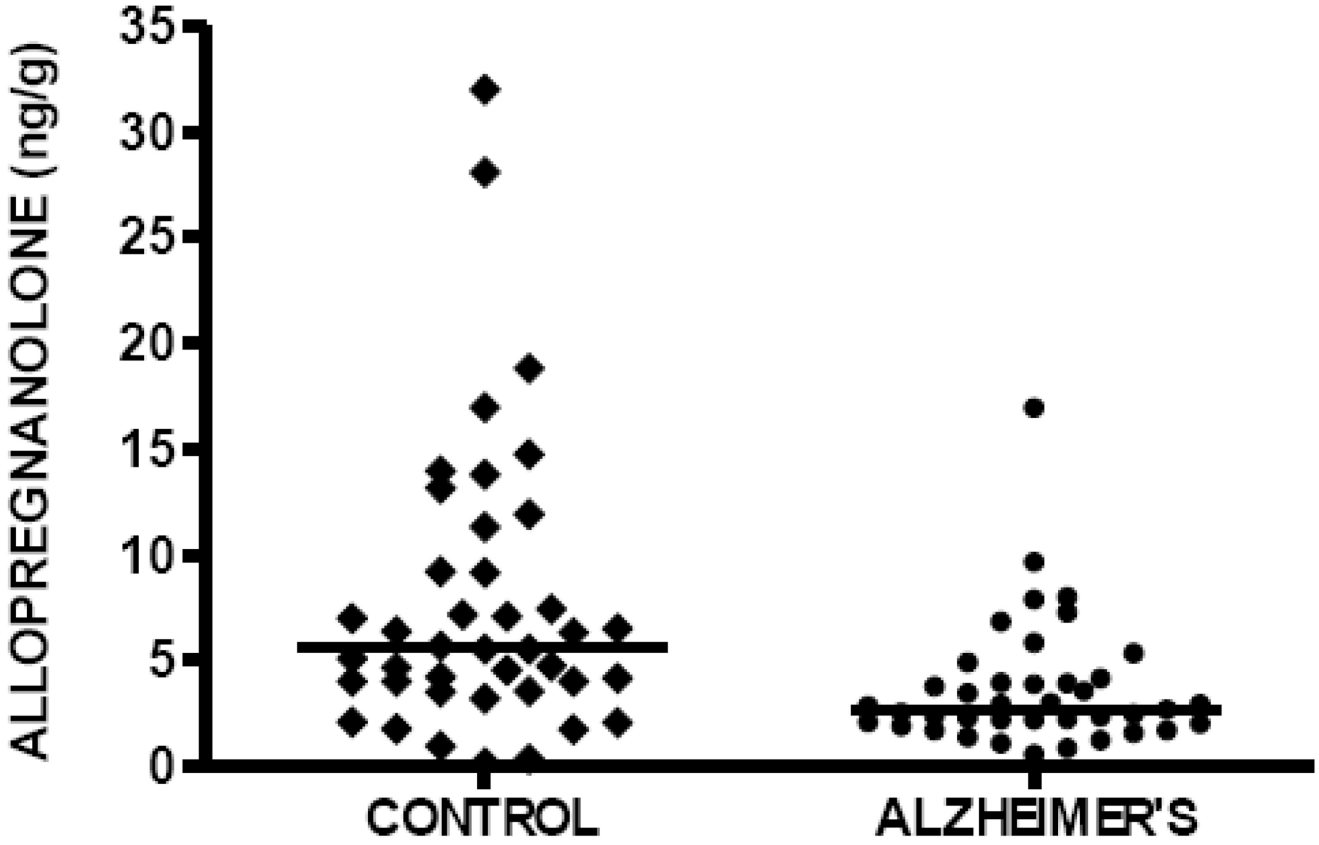

Figure 2.
Allopregnanolone levels in temporal cortex are significantly decreased in subjects with Alzheimer's disease (median 2.68 ng/g, n=40) compared to cognitively intact control subjects (median 5.64 ng/g, n=41), Mann-Whitney p=0.0002.

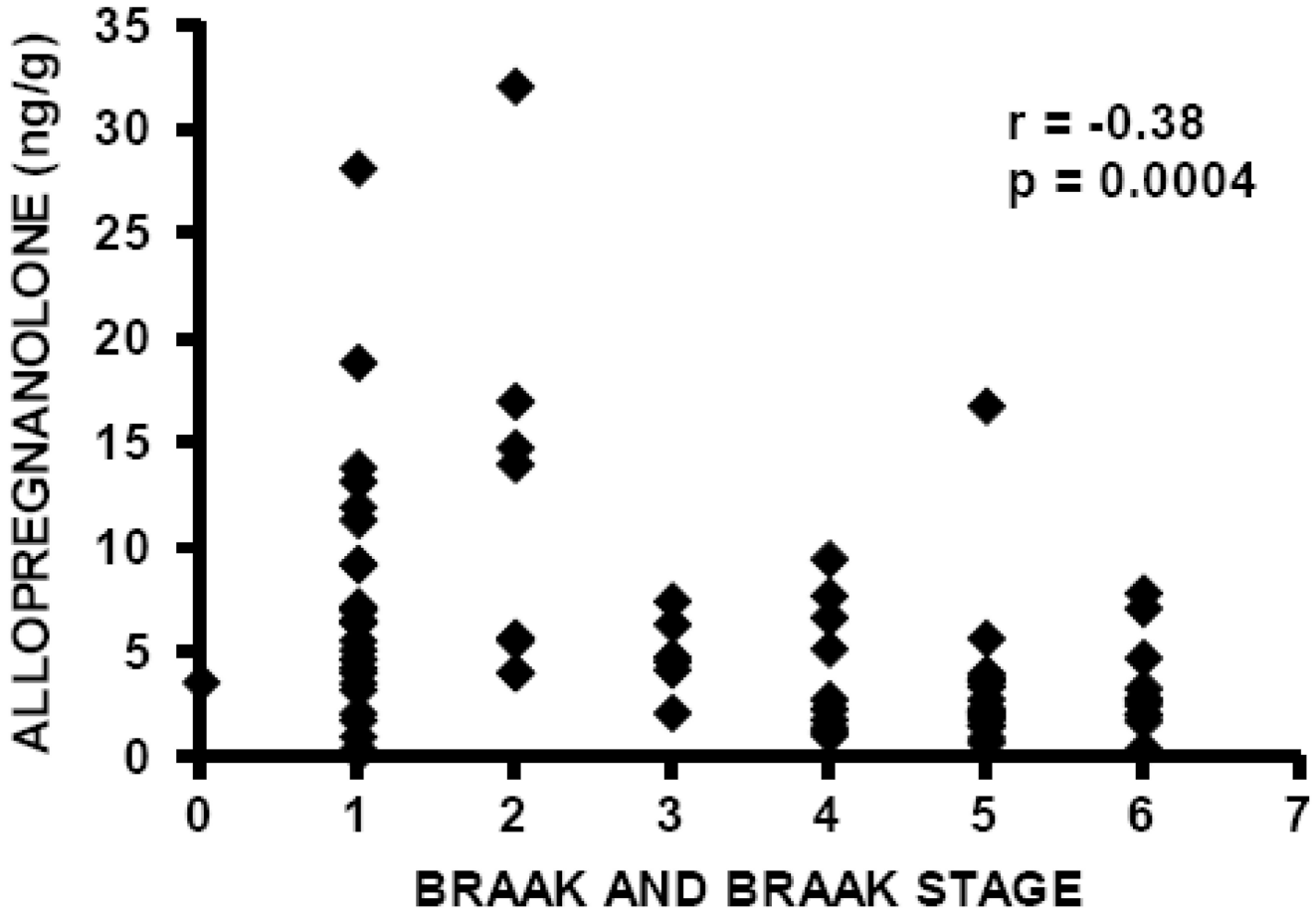

Figure 3.
Allopregnanolone levels in temporal cortex are inversely correlated with neuropathological disease stage (Braak and Braak), Spearman r = −0.38, p=0.0004.

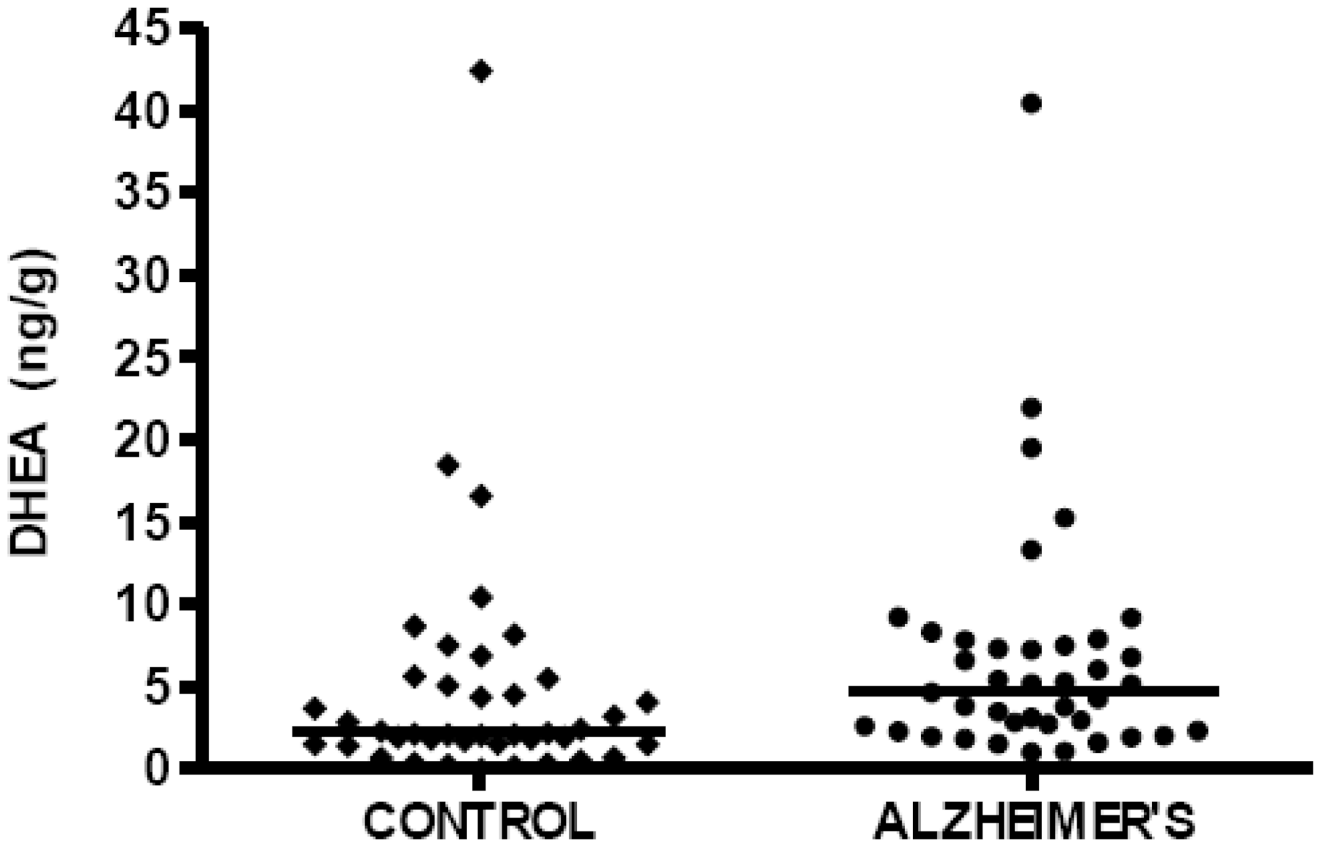

Figure 4.
DHEA levels in temporal cortex are significantly increased in subjects with Alzheimer's disease (median 4.75 ng/g, n=40) compared to cognitively intact control subjects (median 2.23 ng/g, n=41), Mann-Whitney p=0.0185.

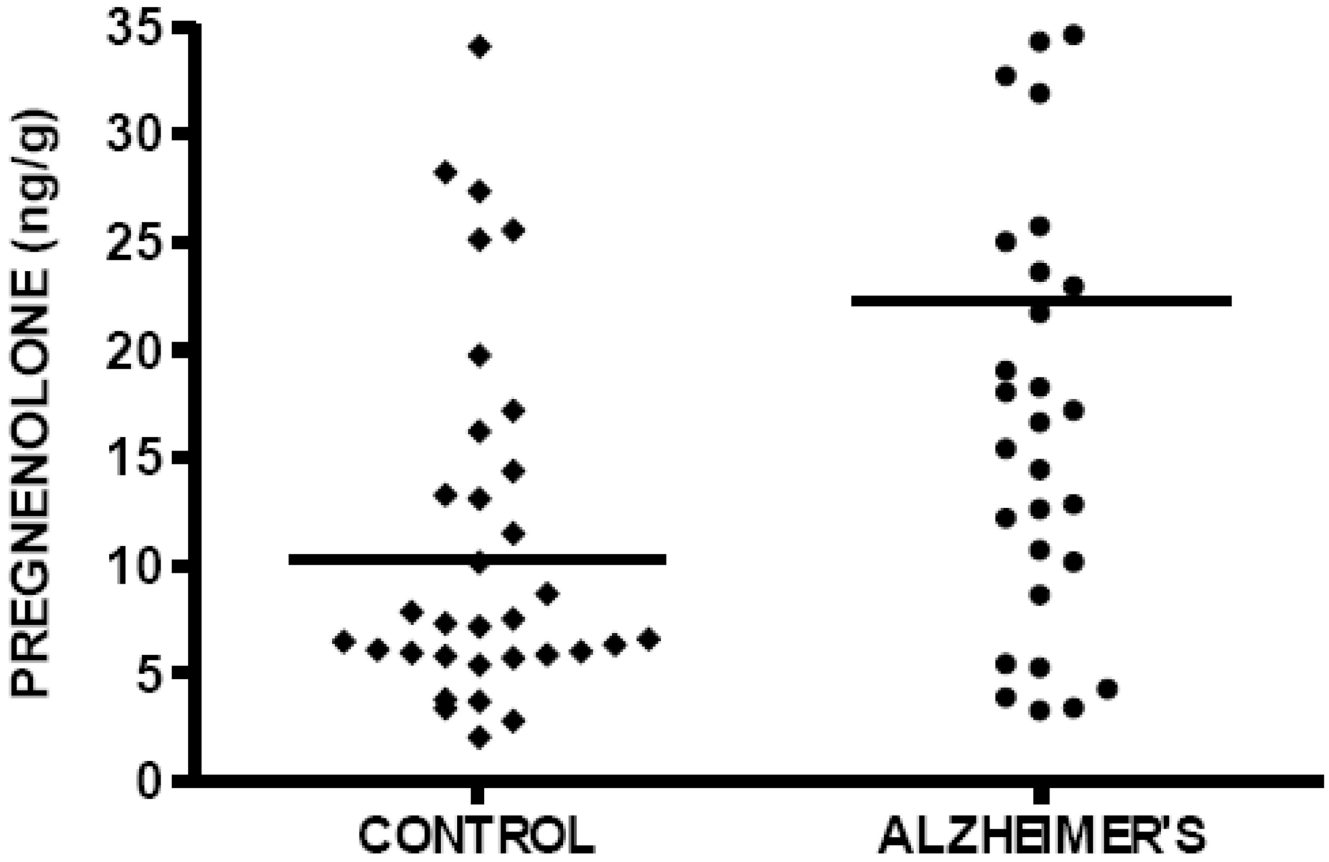

Figure 5.
Pregnenolone levels in temporal cortex are significantly increased in subjects with Alzheimer's disease (median 22.24 ng/g) compared to cognitively intact control subjects (median 10.24 ng/g), Mann-Whitney p=0.022.

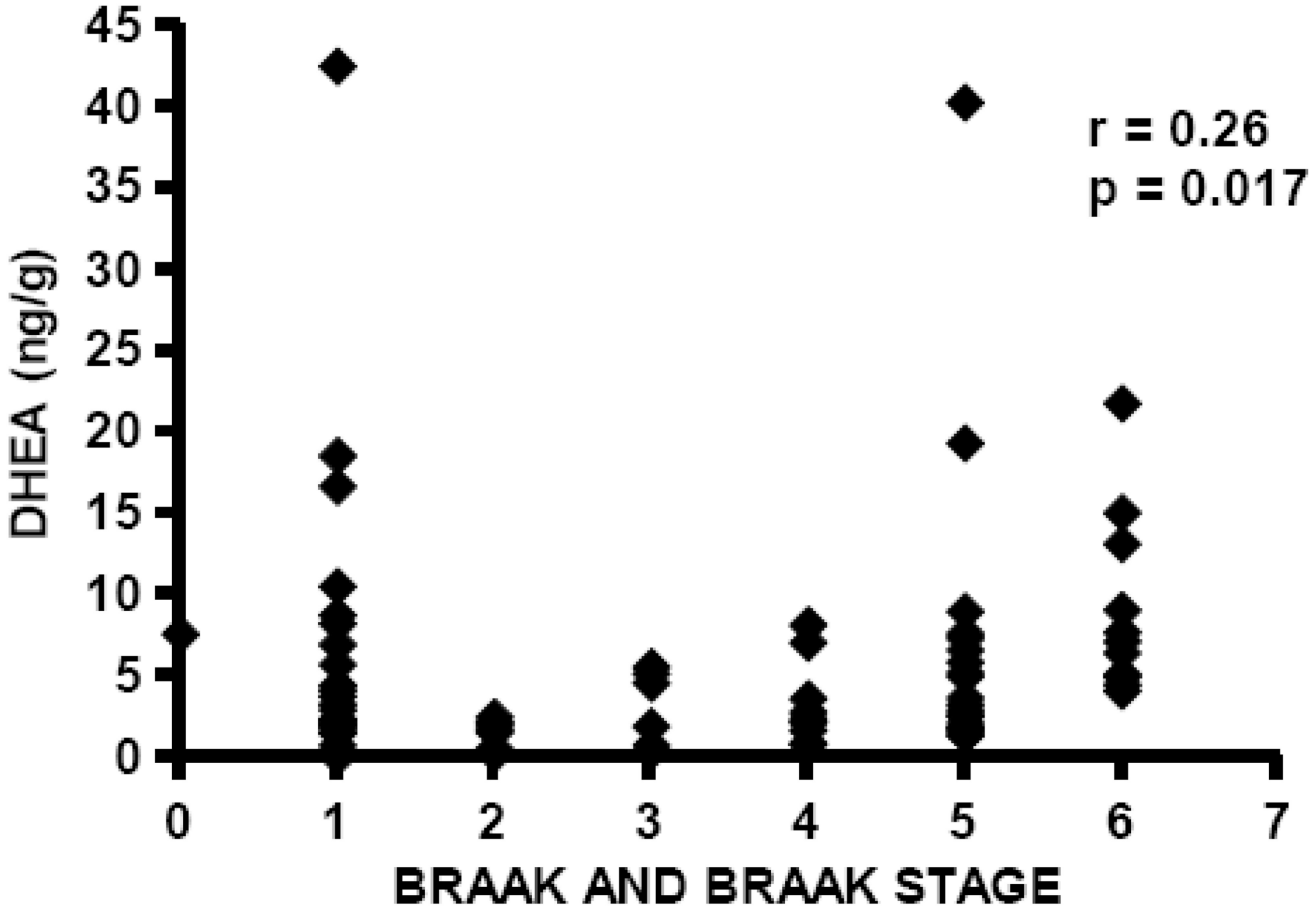

Figure 6.
DHEA levels are positively correlated with neuropathological disease stage (Braak and Braak) in temporal cortex, Spearman r= 0.26, p=0.017.

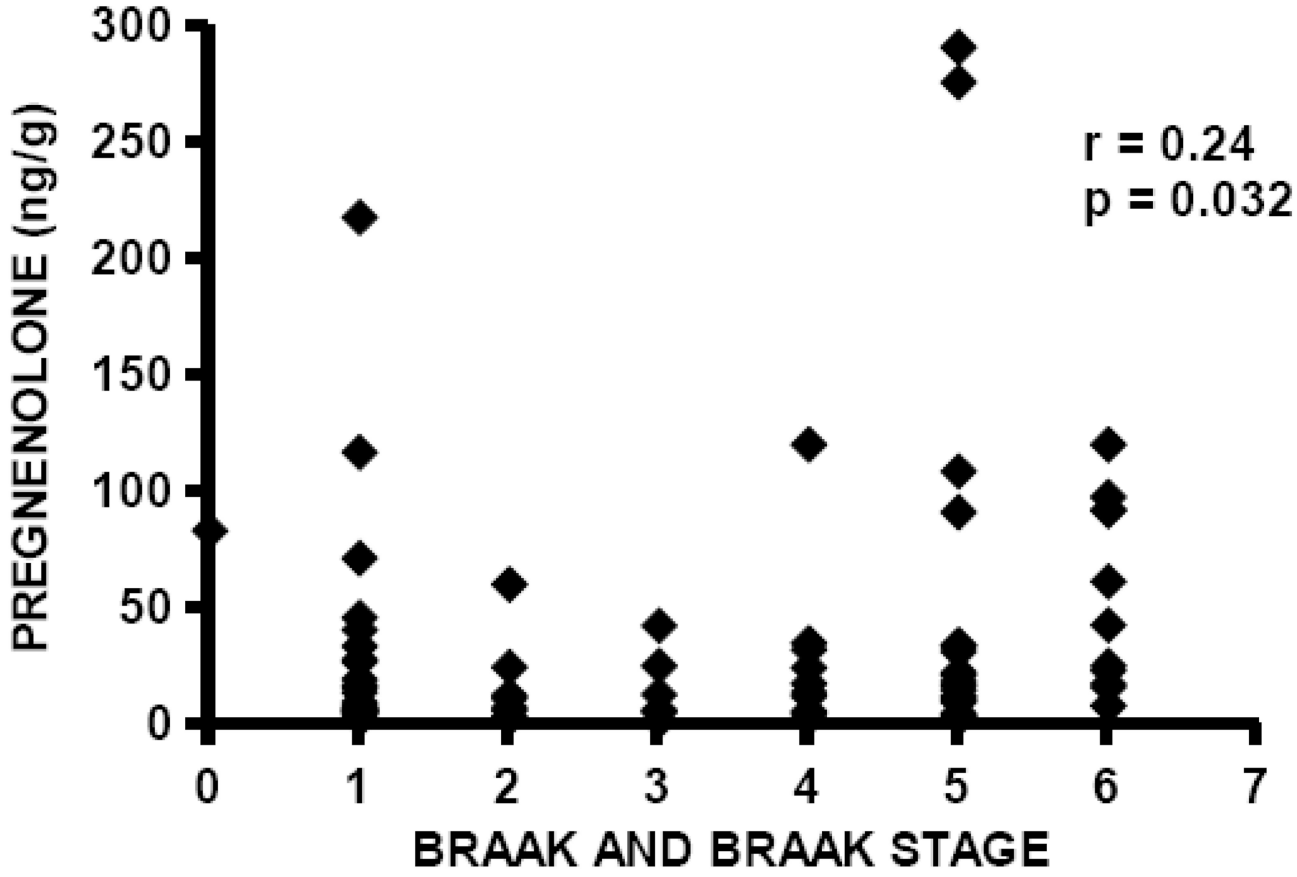

Figure 7.
Pregnenolone levels are positively correlated with neuropathological disease stage (Braak and Braak) in temporal cortex, Spearman r=0.24, p=0.032.

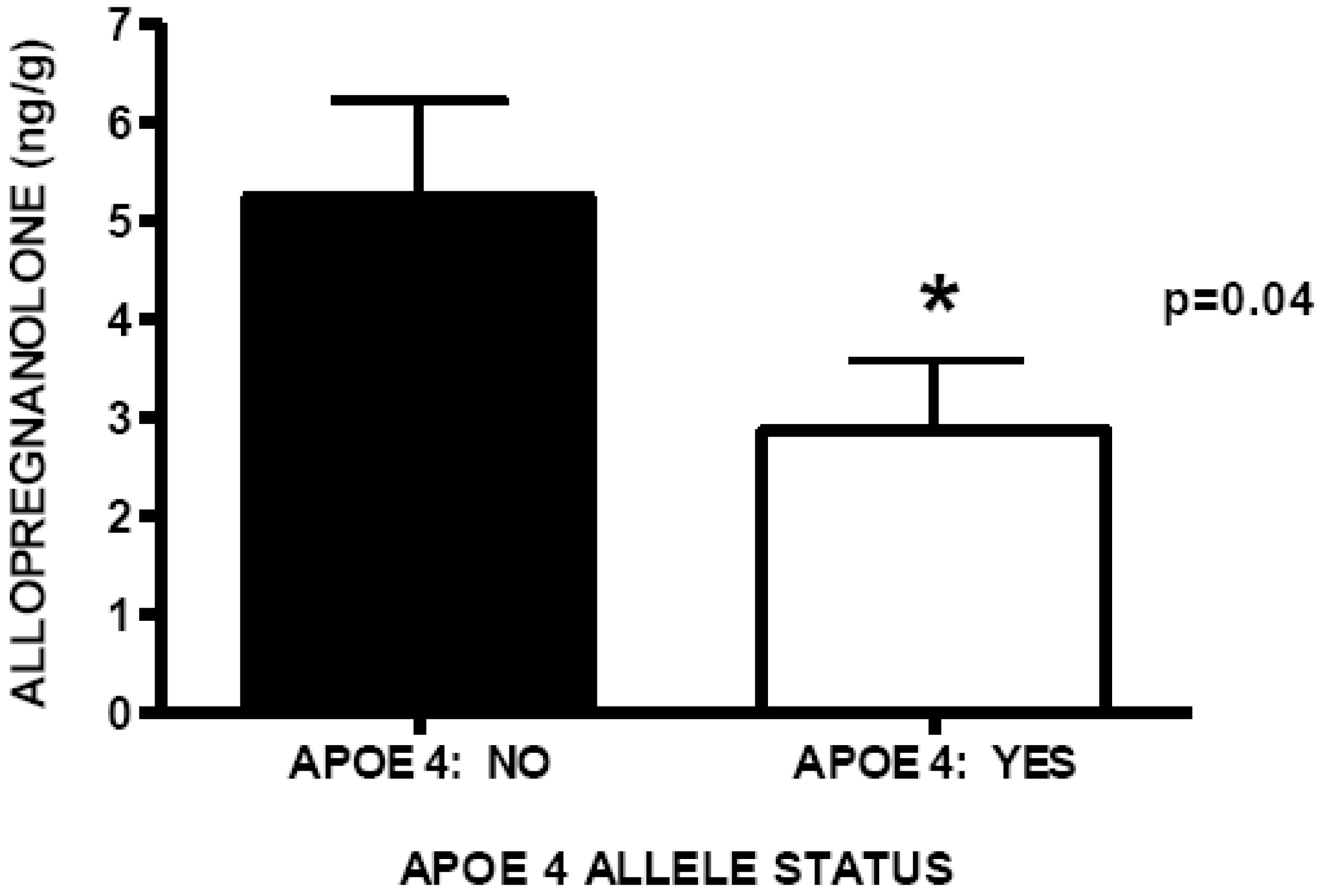

Figure 8.
Allopregnanolone levels in temporal cortex are significantly decreased in subjects homozygous or heterozygous for the APOE4 allele (median 2.86 ng/g, n=36) compared to patients who are not APOE4 allele carriers (5.23 ng/g, n=44), p=0.04.

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Table 1

Patient Characteristics.

Age	Post mortem Interval (min)	Gender	APOE4 Status	Clinical Diagnosis	Cause of Death
75	154	Male	3.4	AD	Unavailable
88	150	Male	4.4	AD	Unavailable
81	965	Male	3.3	AD	Unavailable
66	42	Male	4.4	AD	Acute Bronchopneumonia
82	80	Male	3.4	AD	Pneumonia
68	425	Male	3.3	AD	Cardia Arrhythmia
81	570	Male	3.4	AD	Unavailable
90	120	Male	3.4	AD	Secondary to Carcinoma of the Colon
90	2040	Male	3.4	AD	Pneumonia
74	160	Male	4.4	AD	Acute Aspiration Bronchopneumonia
83	120	Male	3.3	AD	Aspiration Pneumonia
80	390	Male	4.4	AD	Pneumonia
90	1050	Male	3.4	AD	Cardiorespiratory failure
85	587	Male	3.3	AD	End Stage Dementia
71	390	Male	3.4	AD	Aspiration Pneumonia
69	1145	Male	3.4	AD	Aspiration Pneumonia
83	769	Male	3.3	AD	Unavailable
85	120	Male	3.3	Control	Conqestive Heart Failure
75	1133	Male	3.3	Control	Cardiovascular Disease
78	1149	Male	3.3	Control	Sepsis
66	65	Male	3.3	Control	Adenocarcinoma of the Prostate
74	69	Male	3.4	Control	Adenocarcinoma of the Prostate
81	630	Male	3.3	Control	Unavailable
82	195	Male	3.3	Control	Cardiac Arrest
63	420	Male	3.3	Control	Myocardial Infarct

Age	Post mortem Interval (min)	Gender	APOE4 Status	Clinical Diagnosis	Cause of Death
80	255	Male	3.3	Control	Unwitnessed Cardiopulmonary Arrest
40	345	Male	Unknown	Control	Myocardial Infarct
81	433	Male	3.4	Control	Dehydration; GI Hemorrhage
90	461	Male	3.3	Control	Myocardial Infarct
83	550	Male	3.3	Control	Prostatic Carcinoma
90	720	Male	3.3	Control	Unavailable
83	120	Male	3.3	Control	Urinary Bladder Rupture
90	330	Male	3.3	Control	Cardiovascular Disease
90	444	Male	3.3	Control	Myocardial Infarct
87	1110	Male	3.3	Control	Adenocarcinoma of the Lung
79	390	Male	2.4	Control	Pneumonia and Urinary Tract Infection
88	1040	Male	3.3	Control	Myocardial Infarct
90	300	Male	3.3	Control	Unavailable
67	369	Female	4.4	AD	Unavailable
90	114	Female	3.3	AD	Unavailable
70	435	Female	3.4	AD	Unavailable
65	283	Female	3.4	AD	Not determined
82	969	Female	3.4	AD	Unavailable
56	390	Female	3.3	AD	Unavailable
63	715	Female	3.4	AD	Heart Disease
67	69	Female	4.4	AD	Aspiration Pneumonia
85	165	Female	3.3	AD	Acute Pneumonia
79	145	Female	3.4	AD	Cerebrovascular Accident
90	350	Female	3.4	AD	Unavailable
78	120	Female	3.4	AD	Unavailable
81	754	Female	3.4	AD	Acute Bronchopneumonia
81	195	Female	4.4	AD	Unavailable
85	165	Female	3.4	AD	Unavailable

Age	Post mortem Interval (min)	Gender	APOE4 Status	Clinical Diagnosis	Cause of Death
88	574	Female	3.4	AD	Aspiration Pneumonia
59	840	Female	2.3	AD	Cardiorespiratory Arrest
82	112	Female	3.4	AD	Cardiorespiratory Failure
80	309	Female	3.3	AD	Respiratory Failure. Aspiration Pneumonitis, Bronchopneumonia
90	652	Female	3.4	AD	Advanced Alzheimer's Disease
59	255	Female	3.3	AD	Unavailable
90	1230	Female	3.4	AD	Unavailable
49	1051	Female	3.3	AD	Acute Bronchopneumonia
50	1193	Female	2.3	Control	Multiple Myeloma
85	945	Female	3.3	Control	Pneumonia. COPD. Heart Disease
61	249	Female	3.4	Control	Myocardial Infarct
49	698	Female	3.3	Control	Respiratory Failure secondary to Multiple Tumor Emboli within the Lungs
72	180	Female	3.4	Control	Bronchopneumonia secondary to Metastatic Breast Cancer
78	331	Female	3.3	Control	Bronchopneumonia
80	69	Female	3.3	Control	Pancreatic Cancer
57	540	Female	3.4	Control	Myocardial Infarct
90	1380	Female	3.3	Control	Metastatic Carcinoma
90	637	Female	3.3	Control	Pneumonia
90	990	Female	3.4	Control	Unavailable
90	225	Female	2.3	Control	Unavailable
82	930	Female	3.3	Control	Myocardial Infarct
90	240	Female	3.3	Control	Unavailable
67	480	Female	3.3	Control	Colon Cancer
82	487	Female	3.3	Control	Dissecting Aortic Aneurysm
74	283	Female	2.4	Control	Cardiorespiratory Failure
88	1230	Female	2.3	Control	Lung Cancer

Age	Post mortem Interval (min)	Gender	APOE4 Status	Clinical Diagnosis	Cause of Death
72	1800	Female	3.3	Control	Unavailable
90	600	Female	2.3	Control	Unavailable

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Table 2

Neurosteroid Levels (median; ng/g) in Temporal Cortex in Cognitively Intact Control Subjects and Patients with Alzheimer's Disease.

	n	Min	Q1	Med	Q3	Max	P-value
Allopregnanolone							0.0002
Alzheimer's disease	40	0.477	1.954	2.681	3.96	16.84	
Control	41	0.266	3.896	5.644	10.37	32.11	
Pregnenolone							0.022
Alzheimer's disease	40	3.184	12.32	22.24	52.49	291.2	
Control	41	2.148	6.089	10.24	27.91	218.2	
DHEA							0.0185
Alzheimer's disease	40	0.87	2.332	4.751	7.523	40.31	
Control	41	0.103	1.625	2.232	5.409	42.51	

Min, minimum, Q1, lower quartile; Med; median; Q3, upper quartile; Max, maximum.

p<05, Mann-Whitney *U* test statistic