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Alzheimer's disease (AD) is an age-related neurodegenerative disorder associated with progressive memory loss, severe dementia, and hallmark neuropathological markers such as deposition of amyloid-β (Aβ) peptides in senile plaques and accumulation of hyperphosphorylated tau proteins in neurofibrillary tangles. Recent evidence obtained from transgenic mouse models suggests that soluble, non-fibrillar Aβ oligomers may induce synaptic failure early in AD. Despite their undoubted value, these transgenic models rely on genetic manipulations that represent the inherited, familial, but not the most abundant, sporadic form of AD. A non-transgenic animal model that still develops hallmarks of AD would be an important step toward understanding how sporadic AD is initiated. Here we show that starting between 12 and 36 mo of age, the rodent *Octodon degus* naturally develops neuropathological signs of AD, such as accumulation of Aβ oligomers and phosphorylated tau proteins. Moreover, age-related changes in Aβ oligomers and tau phosphorylation levels correlate with decreases in spatial and object recognition memory, postsynaptic function, and synaptic plasticity. These findings validate *Octodon degus* as a suitable natural model to study how sporadic AD may be initiated.

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Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized by the accumulation of abnormally processed proteins in neurofibrillary tangles (NFTs) and senile plaques (1). These lesions are present in both familial and sporadic forms of AD. Familial AD is linked to inherited mutations in AD-related genes and represents a small percentage of AD cases, while sporadic AD represents the vast majority of cases and is not inherited. Results from transgenic mice bearing mutations in *APP*, *Psen1/2,* and *Tau* show synaptic dysfunction in early stages of AD, before overt neurodegeneration (2, 3). More recent studies have underscored a critical role for soluble Aβ oligomers as an early trigger for AD, and they have been associated with memory and neural plasticity loss (4-8).

Although transgenic mice have been extremely useful in elucidating the pathological mechanisms of AD, there have been substantial limitations. Several examples are the absence of tau mutations linked to AD, except for a triple transgenic mouse 3xTg-AD, bearing mutations for *APP*, *Psen1/2,* and *Tau* (9), the inability to develop the whole spectrum of the disease, the overexpression of transgenes into a non physiological scenario, and the fact that the manipulated genes represent only familial, and not sporadic forms of AD (10, 11). It would be highly desirable to have a non-transgenic model of AD to complement those that already exist. Several species naturally develop features of AD with age; however, their usefulness is still limited as they do not exhibit the full spectrum AD-related alterations (12-14). For example the Aβ peptide sequences of *Cavia porcellus* (guinea pig) and *Microcebus murinus* are similar to that of human (15, 16), but the first fails to develop senile plaques and NFTs (15) and experiments examining synaptic function and memory have not been carried out in such models. A promising candidate model for

sporadic AD is the rodent *Octodon degus* (degus) which naturally develops the histochemical hallmarks of AD, including intra and extracellular accumulation of amyloid plaques, tau deposition in NFT (17), along with hippocampal disconnection and brain parenchyma pathology (18). Prompted by these preliminary observations, we examined the neuropathological spectrum of AD in degus. Here we report that degus exhibits an age-related accumulation of soluble Aβ oligomers and tau protein phosphorylation that correlates with cognitive decline in spatial (T-maze) and object recognition (ORM) memory, as well as synaptic and neural plasticity dysfunction. Based on these findings we propose that i) Aβ dodecamers (Aβ*56), may associate with phosphorylated tau proteins, constituting an early candidate for the neural toxicity and synaptic dysfunction that occurs before the appearance of fibrillar forms of Aβ, which are common to familial and sporadic forms of AD and ii) degus is a suitable non-transgenic model of sporadic AD.

Results

The degree of neuropathology was evaluated at the behavioral, synaptic, and molecular level in degus at different ages. This approach allowed us to establish interpretative correlations to identify those animals suffering from AD (See Table S2).

Age-related cognitive decline during aging in *Octodon degus*

We first evaluated memory capacity with ORM and T-maze tests in 6, 12, 36, and 60 month old (mo) degus. The results show no significant difference between 6 and 12 mo or 36 and 60 mo degus and therefore animals were considered as young (6 and 12 mo) and aged (36 and 60 mo) (Fig. 1B and D). In the T-maze, the aged degus showed a decreased

performance compared to young degus (Fig. 1A-B; ANOVA, $p < 0.0001$). In the ORM, aged degus explored less (total time and number of visits) and had longer latency than younger animals (Table S1). Moreover, unlike young degus, aged degus did not show a preference between new and familiar objects (Fig. 1C-D; t-test $p < 0.0001$). In general, we observed an age dependent decline in memory performance beginning at 36 mo and maintained through 60 mo (Fig. 1B).

Selective postsynaptic dysfunction induces impairments in synaptic transmission and plasticity.

We next examined the synaptic basis of these learning deficits by evaluating the strength and plasticity of the CA3-CA1 synapses in hippocampal slices prepared from behaviorally tested individuals. Basal excitatory synaptic transmission was reduced in aged degus compared to young degus (Fig. 2A1; ANOVA, $p < 0.0001$). Importantly, the fEPSP slopes, but not fiber volley amplitude, were significantly reduced in aged animals (Fig. 2A2-A3; repeated measures ANOVA, $p < 0.0001$). This is consistent with findings reported in AD mouse models overexpressing mutant forms of APP (19, 20) (see (21) for a review). The reduced transmission could be due to a reduced postsynaptic responsiveness or to a reduced probability of neurotransmitter release. We ruled out the latter possibility because no difference in the paired-pulse facilitation ratio was observed (Fig. 2B1-B2; 2-way ANOVA, $p > 0.05$). To confirm the likely postsynaptic basis for the reduced fEPSPs, we recorded AMPA receptor (AMPAR)-mediated miniature EPSCs (mEPSCs). Consistent with previous reports in AD models (22, 23), we found that the amplitude but not the frequency of mEPSCs was significantly reduced in aged degus (Fig. 2C1-C3; t-test, $p < 0.01$),

suggesting a change in AMPAR function or number. The electrophysiological analysis indicated that aging preferentially affects postsynaptic processes in degus. Therefore, we further assessed the integrity of pre- and postsynaptic elements by measuring the levels of critical synaptic proteins extracted from hippocampal slices by Western blot. We found a selective reduction in PSD-95, AMPAR subunit GluR2, and NMDA receptor (NMDAR) subunit NR2B expression (Fig. 3A-B; ANOVA, $p < 0.05$), but not of synaptophysin in accordance with the observation that measures of presynaptic function (FV and PPF) are not affected in aged degus (Fig. 2A2 and B1). These data support the observation that postsynaptic integrity is more vulnerable in aged degus.

Next we assessed whether synaptic plasticity was affected at the CA3-CA1 synapses of the aged degus. We measured long-term potentiation (LTP) induced with theta-burst stimulation (TBS) and long-term depression (LTD) induced with paired pulse-low frequency stimulation (ppLFS). The LTP amplitude in aged degus was significantly decreased after 60 min compared to that of young degus (Fig. 4A; 2-way ANOVA, $p < 0.0001$), whereas LTD was slightly but significantly increased in aged degus compared to young (Fig. 4B, 2-way ANOVA, $p < 0.0001$). Prompted by the fact that neural plasticity was similar in the 36 and 60 mo age groups, suggesting a decrease in the progression of neurodegeneration, we examined an additional group of 72 mo degus. At this age, LTP continues to decrease and LTD to increase (Fig. S1A) indicating a further progression of the neuropathology associated with AD.

Synaptic plasticity measurements were recorded in behaviorally characterized degus, raising the possibility that synaptic modifications induced during the learning tasks might have affected the subsequent induction of LTP and LTD in slices (24). However, the

magnitude of LTP and LTD were similar (Fig. S4C-D) in slices from naïve and trained degus, indicating that the effects of previous behavioral testing were minimal.

Increased soluble Aβ oligomers and tau phosphorylation in aged degus.

Growing evidence establishes that soluble Aβ oligomers are involved in synapse and memory impairments in AD (5-7). To identify the nature of different types of Aβ species in hippocampal extracts from young and aged degus, we used a specific mouse anti- Aβ peptide 4G8, followed by immunoblotting (7). Both young and aged degus show similar forms of small Aβ peptides, such as trimers (~12 KDa), tetramers (~16 KDa), and hexamers (~27 kDa), while aged degus displayed higher levels of Aβ dodecamers (~56 kDa) (Fig. 5A1-A2). The presence of these Aβ dodecamers in the hippocampus was confirmed by a slot blot assay using the specific antioligomeric antibody A11, which detects soluble amyloid assemblies larger than 40 kDa (25). Aged degus showed greater levels of larger Aβ oligomers (A11) when compared to young degus (Fig. 5B).

The number and localization of NFT, but not senile plaques, has been correlated with levels of dementia in AD patients (26). In order to search for the presence of NFTs, we measured tau phosphorylation at sites known to be present in paired helical filaments (PHFs). The PHF-1 antibody detects tau phosphorylation at serine residues 396 and 404 (27). Aged degus show an increase in the phosphorylation levels detected by this antibody compared to young degus (Fig. 5C). A similar result was obtained by using AT8 antibody, which detects tau phosphorylation at serine 202 and threonine 205 (28), Tau phosphorylation at these sites was only slightly greater in aged degus, including 72 mo degus (Fig. S1B-C), compared to young degus (Fig. 5C). The increase in tau phosphorylation was not simply

due to an increase in tau protein levels. Fig. S1 (B-C) shows that the total tau level (Tau-5) remains unchanged up to 72 mo. Furthermore, pathological phosphorylated tau begins to appear first in the cortex and then in the hippocampus, where neurons containing hyperphosphorylated tau were preferentially detected in aged degus (Fig. 7 and S5, also see Fig. 1 in (17)).

Increased levels of soluble Aβ oligomers and tau phosphorylation correlate with behavioral impairment and reduced synaptic plasticity.

Table S2 shows that the 12mer dodecamer (Aβ*56) is highly negatively correlated with LTP magnitude ($r^2 = 0.31$, $p = 0.0012$; Fig. 6A), and performance in Tmaze ($r^2 = 0.24$, $p = 0.0048$; Fig. 6B), and ORM ($r^2 = 0.34$, $p = 0.0006$; Fig. 6C) tasks. Similarly, higher levels of PHF-1 correlate with lower levels of LTP ($r^2 = 0.28$, $p = 0.0020$, Fig. 6D), ORM ($r^2 = 0.37$, $p = 0.0003$; Fig. 6F), and to a lesser degree with T-maze ($r^2 = 0.19$, $p = 0.0138$; Fig. 6E) performance. Comparable correlations were obtained when A11 and AT8 were compared with LTP, T-maze, and ORM performance (Fig. S2). Further analysis using Principal Component Analysis is presented in supplementary information.

Although these results suggest a similar time course and a plausible causal link between the increase in soluble Aβ oligomers, phosphorylated tau, and the incidence of synaptic and cognitive alterations, we cannot rule out the possibility that insoluble amyloid fibrils also participate in this cascade of neurotoxic events. For this reason, we analyzed the presence of amyloid deposition by immunohistochemistry and Thioflavine S (ThS) staining (Fig. 7 and S5). Amyloid plaques were almost absent in young degus and began to appear in the cortex of aged degus at 36-60 mo, whereas plaques did not appear in the hippocampus until

over 60 mo (Fig. S5). Just as with ThS-positive plaques, extracellular Aβ immunoreactivity (Fig. 7I) and pathological phosphorylated tau was first observed in the cortex at 36 mo, and later in the hippocampus after 60 mo (Fig. 7J).

Results here indicate that during aging, degus develop a neurodegenerative AD-like condition. In the initial stages, increased Aβ oligomers and phosphorylated tau levels could explain the impairments in learning, memory, and neural plasticity capacities; whereas in later stages (after 72 mo), progressive deposition of plaques and tangles, likely leading to neurodegeneration, occurs which could aggravate or accelerate the symptomatology of AD in degus (Fig. 7, S1, S5).

Throughout this study we noted that the behavioral, functional, and molecular alterations did not proceed at a uniform pace during aging. Rather, most of the changes occurred between 12 and 36 mo. To identify the most critical factors involve in the progression of AD, we performed a Principal Component Analysis (PCA) (Table S2; variables with correlation ≥ 0.50: LTP, Tmaze, ORM, Aβ*56, PHF1, GluR2, and NR2b). Interestingly, the plot in the space of the first two principal components (PC1 vs PC2) clearly segregates two different classes of individuals: young and aged degus (Table S4, Fig. S3), supporting the importance of the variables examined here. Finally, it is important to note that although most of the deficits develop between 12 and 36 months, the changes are not homogeneous in this population. About 25% of individuals aged 36-months exhibit "unimpaired" performance either in the spatial or the object recognition memory test (59). This variability would be expected for a group of non-transgenic animals where the genetic background comes from a controlled but natural population.

Discussion

Synaptic and cognitive dysfunction in AD models has revealed significant impairments before neurodegeneration becomes evident (19, 20, 22, 23). We show here a clear correlation between high levels of Aβ*56 oligomers and tau phosphorylation with the reduction of synaptic strength and plasticity (Fig. 6 and S3). However, the exact mechanism by which Aβ oligomers or phosphorylated tau might impair synaptic function is still under debate. Some studies have reported that the addition of Aβ oligomers is sufficient to impact synaptic function *in vitro* (5, 29, 30) as well as alter cognitive function (7, 31). Other studies provide evidence that chemical reactions occurring during the process of Aβ aggregation produce toxic species like reactive oxygen species (32, 33). In that context, degus could provide a valuable "natural model" to test toxicity mechanisms.

Recent evidence suggests that soluble Aβ oligomers (also referred to as ADDLs) may induce synaptic failure as an early event related to memory deficits in AD (6, 34). Indeed, it has been observed that soluble Aβ particular affects synapses by selectively targeting postsynaptic components (34, 35). More recently it has been reported that cellular prion protein (PrP^C) functions as a receptor for Aβ oligomers (36). Once bound to the membrane, these oligomers tend to accumulate at excitatory synapses forming clusters with metabotropic glutamate receptors (mGluR5) causing impairments in synaptic plasticity (37). Many different lengths and conformational states of Aβ peptide are generated during its biosynthesis, including highly mobile soluble Aβ oligomers, pre-fibrillar, and fibrillar aggregates. These diverse assemblies have been associated with the disruption of memory and synaptic plasticity. For instance, dimers (8, 38), trimers (39) and dodecamers (7), derived from diverse sources (including chemical synthesis, transfected cells, and mouse

and human AD brains), potently impair synaptic plasticity and memory. However, it has been showed that Aβ*56, compared to others assemblies, has the greatest effect on memory (7, 40, 41) and has been proposed to be the key neurotoxic non-fibrillar assembly in AD because it is highly stable and prone to aggregate (42). In the same way, we observed in degus a stronger inverse relationship between increasing levels of Aβ*56 and LTP, T-maze, and ORM performance, which becomes more severe during aging (Fig. 6). Strikingly, aged degus also show an increase in the levels of phosphorylated tau (Fig. 5C, S5), suggesting a functional link between Aβ processing and tau phosphorylation, since phosphorylated tau residues also negatively correlate with LTP and cognitive impairments (Fig. 6). Several reports have shown that in the hippocampus of mice, an increase in soluble Aβ by local administration increases the level of phosphorylated tau proteins, producing cognitive impairments (43-45). Furthermore when both proteins decrease, a recovery of cognitive abilities was observed (44). Interestingly, direct interaction between tau proteins and Aβ peptides induces tau aggregation and hyperphosphorylation (45). Furthermore, it has recently been shown that Aβ oligomers cause missorting and phosphorylation of tau (46), microtubule destabilization, and axonal transport disruption (47). On the other hand, Aβ-induced impairments in LTP are mediated by tau phosphorylation suggesting that tau proteins are one element required for the synaptotoxic effects of Aβ oligomers (48). Age-related reductions in spontaneous and evoked AMPAR-mediated currents have also been observed in AD models, which are attributed to a reduction in the number of these receptors (22, 23). We did not observe differences in the frequency of mEPSCs, suggesting that in 60 mo degus the number of functional synapses is not reduced. However, a possible explanation for the observed decrease in mEPSCs amplitude is that the content of AMPARs

available for trafficking is impaired in aged degus. Certainly, Aβ peptides prevent recruitment and anchoring of AMPARs to the postsynaptic compartment by reducing CAMKII activation and distribution as well as PSD-95 levels (49-51). Together, these results suggest that postsynaptic compartments are more susceptible to the effects of Aβ peptides than are presynaptic elements, considering that both PSD-95 and glutamate receptors were reduced in aged degus (Fig. 2 and 4). However, presynaptic mechanisms cannot be totally excluded since APP (23), Aβ (52), and presenilin (1/2) (53) have been reported to be localized presynaptically.

An interesting comparison can be made between degus and the 3xTg-AD mice (9). In both models, the decline in synaptic plasticity can be associated with an increase in Aβ peptides before an increase in tau phosphorylation occurs. Since this process occurs naturally in degus, it represents a unique opportunity to test for physiological mechanism and rescue therapies during sporadic AD.

Another motivating comparison here is with *Microcebus murinus*, a small nocturnal primate living 8-14 years old in captivity in which 20% of elderly adults show neurodegenerative hallmarks of spontaneous AD, including brain amyloid plaques, tau pathology, decrease of ACh neurons, and behavioral failures with loss of sensory and cognitive functions (review in (16)). As in microcebus, 72 mo degus develops a discrete number of Aβ deposits, which are first detectable in the cortex and subsequently in the hippocampus (Fig. 7 and S5, also see Fig.1 in (17)). We have also established changes in neural plasticity (LTP, LTD) in degus that correlate with the presence of soluble Aβ oligomers and phosphorylated tau proteins.

A recurrent and difficult question to address while studying neurodegeneration is its close association with the natural aging process. For example, aged rats show a modest decrease in basal synaptic transmission, NMDAR-mediated response, and deficits in synaptic plasticity (54-56) similar to the decreases in both synaptic transmission and plasticity (Fig. 2 and 3) seen in degus. However, aged rats do not show a decrease in spatial (T-maze) (56) or ORM performance (57, 58), which is clearly different from the performance observed in degus (Fig. 1).

Together, our findings suggest that degus provides a strong and naturalistic model to study early neurodegenerative process associated with sporadic AD. It is worth mentioning that degus can live around 9-10 years in captivity, so the present work represents only half of a degus lifespan. Finally, while the precise mechanism remains unclear, our data are consistent with the idea that soluble Aβ oligomers at pre-fibrillar stages can act as toxic ligands at postsynaptic compartments, driving the synaptic and memory disruption observed in early AD models.

Materials and methods

See SI Material and Methods for detailed information about all techniques used here.

Animals: *Octodon degus* were obtained from a breeding colony at the Animal facility of the Universidad de Valparaiso (Approved Animal Welfare Assurance NIH A5823-01).

Protocols: We submitted the different groups of degus to a complete characterization including, behavioral tests, electrophysiological recordings and biochemical measures. All of animals, regardless of age, received the same manipulations: before evaluating their cognitive capacity, we first habituate the animals to an open field over 5 consecutive days;

next, degus were submitted to an object recognition memory test for 5 days and then to a T-maze test for 17 days. Once the behaviorally characterization was complete, degus were sacrificed to obtain hippocampal slices from which to study synaptic transmission and plasticity. After completion of the electrophysiological experiments, hippocampal slices were immediately frozen for biochemical characterization. We later collected all the tissue to obtain homogenates from the hippocampus to quantify different proteins levels by immunoblotting.

Behavioral tests. Object recognition memory (ORM) was assessed with an open field arena made of black plexiglass (50 cm × 40 cm × 63 cm) during 5 days. Each ORM session consisted of three phases (180 sec each one): (i) Familiarization where degus explored a pair of identical objects; (ii) Retention where the degus is removed for object cleaning and changing; (iii) Recognition where degus explored a pair of different objects: a familiar object (FO) (extra copy of familiar object) and a novel object (NO). To quantitate OMR, a preference index (PI) was calculated by computing PI = NO / NO+FO. Spatial working memory was assessed in a T-maze task using a training protocol to search for a reward during 12 consecutive days. Each session consisted of 10 trials composed of three parts (60 sec each): (i) forced choice memory learning, with no food reward and where one arm remained closed; (ii) retention time, where the animal was removed for cleaning and the closed arm was opened; (iii) free choice for memory recognition, where the two arms are open and the reward was placed in the previously closed arm. A correct response corresponded to a visit to the closed arm during (i), in which case the animal was rewarded with a sunflower seed.

Electrophysiology: Extracellular and whole-cell patch-clamp recordings were performed in hippocampal slices from behaviorally characterized degus as described (54, 55).

Immunoblotting: Proteins were run on gradient, denaturing gels, blotted, and probed with appropriate antibodies.

Histology: PFA-fixed brains were sectioned into 20µm slices and free-floating slides were processed following immunohistochemical and ThS staining procedures.

Statistics: All data are presented as mean ± standard error or deviation of the mean (S.E.M. or S.D.). Data analysis was carried out using Prism software (GraphPad Software Inc).

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Figure Legends

Fig. 1. Age-dependent decline in cognitive performance in *O. degus*. A: Average number of correct choices per day in the T-maze test. Two-way ANOVA ($F_{(11,33)} = 34.59$, * $p < 0.0001$) followed by Bonferroni post-hoc test ($p < 0.05$) on the last 3 days for aged (36-60 mo) versus young (6-12 mo). B: Average correct choices at the end of the experimental phase from 6 mo (white), 12 mo (gray), 36 mo (blue) and 60 mo (red) degus. One-way ANOVA ($F_{(3,26)} = 6.509$, *$p = 0.002$) followed by Tukey´s post-hoc test ($p < 0.05$). C: Preference index for object recognition. Paired two-tailed t-test (*$p < 0.01$) novel versus familiar objects. D: Average exploration time for novel vs familiar objects. Paired two-tailed t-test (*$p < 0.01$) versus familiar objects. The values in parentheses indicate the number of animals.

Fig. 2. Altered synaptic transmission and postsynaptic deficits in the Schaffer Collateral–CA1 pathway. Representative traces of fEPSP at different stimulus intensities from 6 mo (white), 12 mo (gray), 36 mo (blue) and 60 mo (red) degus. Scale bars: 1mV, 10 ms. A1: AMPAR mediated input-output curves, from 6 mo (white), 12 mo (gray), 36 mo (blue) and 60 mo (red) degus. One-way ANOVA ($F_{(3,58)} = 157.3$, *$p < 0.0001$) followed by Tukey´s post-hoc test ($p < 0.05$). A2: Relationship between FV amplitude or A3: fEPSP slope and stimulus intensity from 6 mo (white), 12 mo (gray), 36 mo (blue) and 60 mo (red) degus. Repeated measures ANOVA ($F_{(15,45)} = 61.06$, *$p < 0.0001$) followed by Bonferroni post-hoc test ($p < 0.05$). B1: Normal paired-pulse facilitation between groups. B2: Representative traces at interstimulus intervals of 50 ms are shown. Scale bars: 1 mV, 20 ms. C1: Amplitudes and frequencies of AMPAR mediated mEPSCs from young (white) and aged (red) degus. C2: Cumulative probability plots for mEPSC amplitude size. Insert, representative superposed events, Calibration: 5pA, 20ms C3: Representative traces of mEPSCs are shown. Scale bars: 20pA, 2 s Calibration: 1mV, 10ms. Unpaired two-tailed t-test (*$p < 0.01$) young versus aged. The values in parentheses indicate the number of hippocampal slices (left) and the number of animals (right) used.

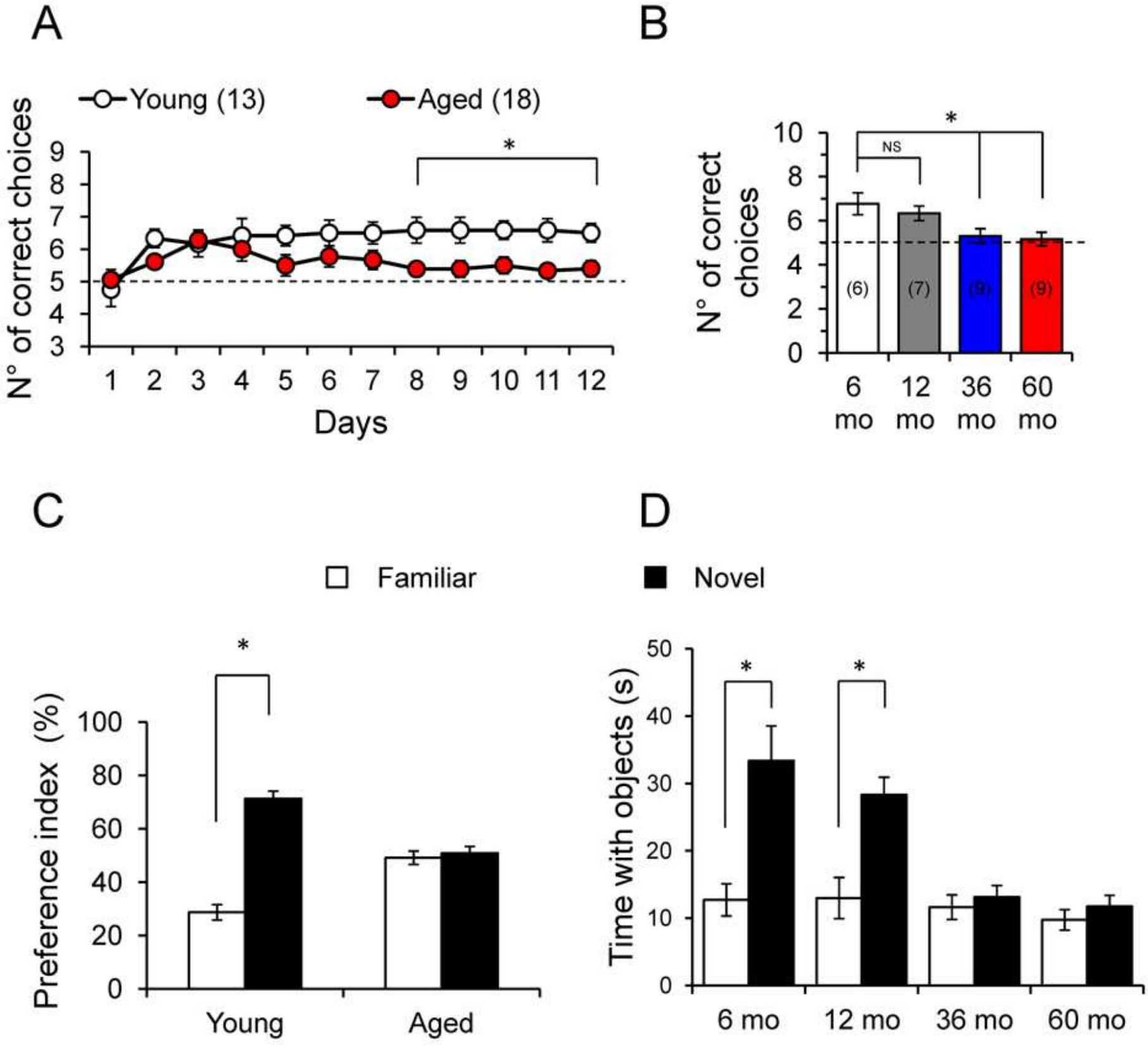
Fig. 3. Postsynaptic proteins affect synapse during degus aging. A: Representative blot of synaptic proteins from hippocampus extracts (age indicated above lanes). Arrows indicate respective migration positions. B: Relative levels of synaptophysin (SYP), PSD-95, GluR2-AMPAR subunit and NR2b-NMDAR subunit in the hippocampus from 6 mo (white), 12 mo (gray), 36 mo (blue) and 60 mo (red) degus. Mean values of synaptic proteins are relative to β-tubulin levels. One-way ANOVA ($F_{(3,20)} = 6.626$, *$p = 0.0027$ for PSD-95), ($F_{(3,20)} = 3.968$, *$p = 0.0227$ for GluR2) and ($F_{(3,21)} = 2.662$, *$p = 0.0745$ for NR2b) followed by Tukey´s post-hoc test ($p < 0.05$) for young (6-12 mo) vs aged (36-60 mo).

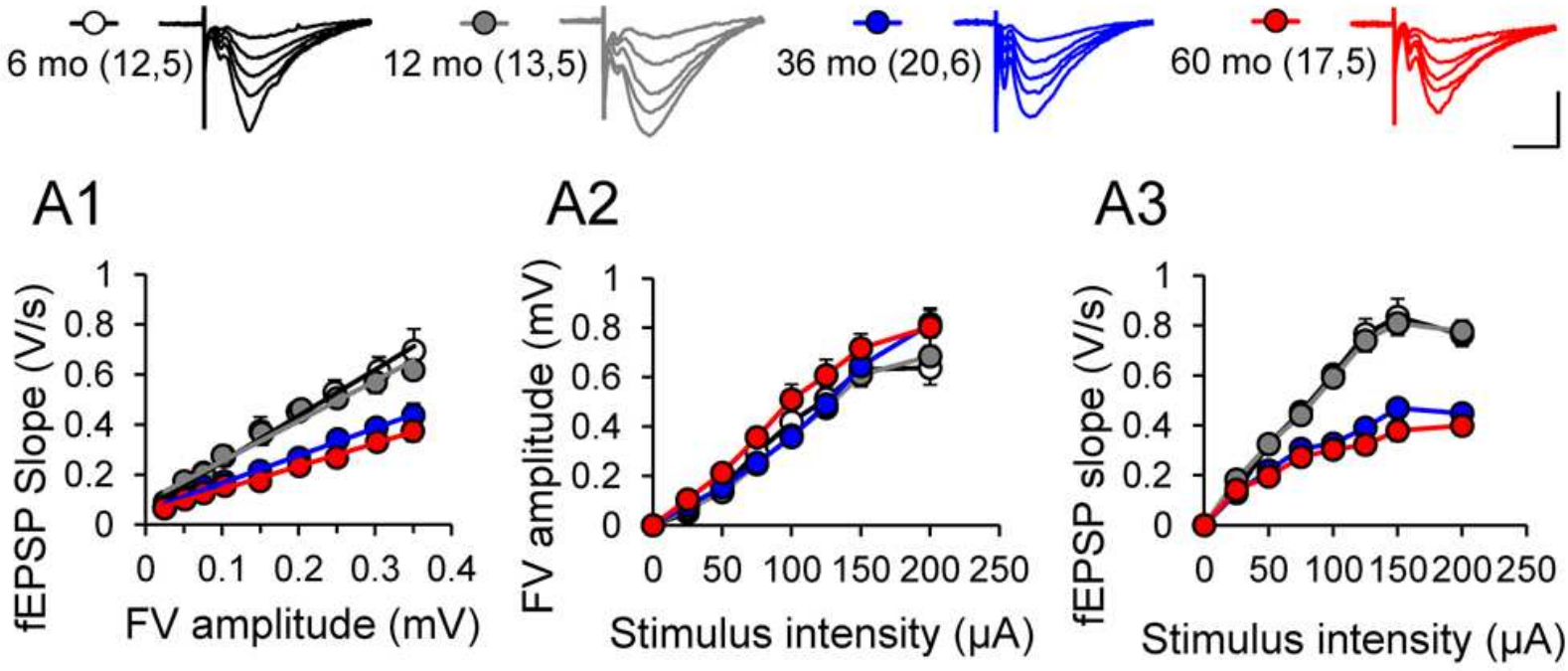
Fig. 4. Impaired hippocampal synaptic plasticity in aged *Octodon degus*. A: TBS-induced LTP in Schaffer Collateral–CA1 synapse. Traces shown to the right are representative fEPSPs recorded 1 min before (1) and 60 min after (2) TBS. LTP protocol was delivered at the time indicated by the arrow. Averaged LTP magnitude during the last ten minutes of recording for 6 mo (white), 12 mo (gray), 36 mo (blue) and 60 mo (red) degus is also shown. Two-way ANOVA ($F_{(1,19)}$ = 1841, *p < 0.0001) followed by Bonferroni post-hoc test (p < 0.05) on the last 10 min for aged versus young. B: ppLFS-induced LTD in Schaffer Collateral–CA1 synapse. Traces shown to the right are representative fEPSPs recorded 1 min before (1) and 60 min after (2) ppLFS. LTD protocol was delivered at the time indicated by the horizontal open bar. Averaged LTD magnitude during the last ten minutes of recording for 6 mo (white), 12 mo (gray), 36 mo (blue) and 60 mo (red) degus is also shown. Two-way ANOVA ($F_{(1,19)}$ = 435.6, *p < 0.0001) followed by Bonferroni post-hoc test (p < 0.05) on the last 10 min for aged versus young. Scale bars: 1mV, 10ms. The values in parentheses indicate the number of hippocampal slices (left) and the number of animals (right) used.

Fig. 5. Accumulation of large soluble Aβ oligomers and tau phosphorylation during aging. A1: Representative blot for amyloid oligomers using anti-Aβ peptide antibody 4G8. Arrows indicate respective migration positions of hexamers (6-mer), nonamers (9-mer) and dodecamers (12-mer). Synthetic Aβ42 peptide was used as size marker and positive control (right lane). A2: Identification and relative levels of different Aβ oligomeric associations in hippocampal extracts from 6 mo (white), 12 mo (gray), 36 mo (blue) and 60 mo (red) degus. One-way ANOVA ($F_{(3,21)}$ = 17.21, *p < 0.0001 for 6-mer) and ($F_{(3,19)}$ = 5.439, *p=0.0026 for 12-mer) followed by Tukey´s post-hoc test (p < 0.05) for aged versus young. B: Relative levels of soluble Aβ oligomers (Insert, representative slot blot from hippocampal extracts using anti-oligomeric antibody A11). One-way ANOVA ($F_{(3,19)}$ = 5.439, p = 0.0072) followed by Tukey´s post-hoc test (p < 0.05) for aged (36-60 mo) versus young (6-12 mo). C: Determination of phosphorylated tau protein levels using PHF-1 and AT8 antibodies. (Insert, representative blot for PHF-1, AT8 and β-actin). One-way ANOVA ($F_{(3,20)}$ = 23.21, *p < 0.0001, for PHF-1) and ($F_{(3,20)}$ = 5.95, *p = 0.0059, for AT8) followed by Tukey´s post-hoc test (p < 0.05) for aged versus young. The values in parentheses indicate the number of animals used.

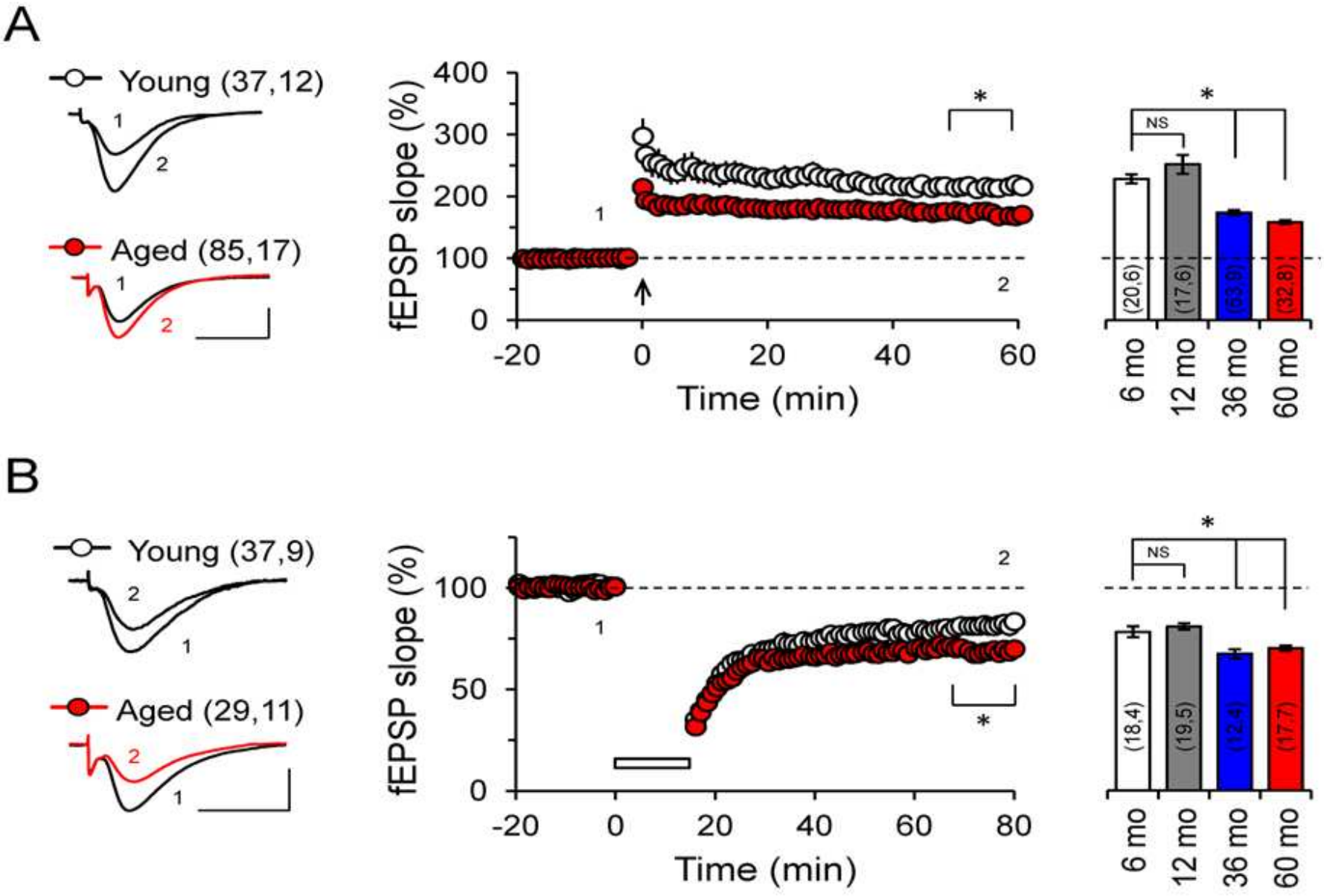
Fig. 6. Large Aβ oligomers and tau phosphorylation correlate with LTP and memory impairments. Relationships between LTP magnitude (A), T-maze (B), ORM (C) and soluble dodecamer (Aβ*56) levels for young and aged degus. Relationships between LTP magnitude (D), T-maze (E), and ORM (F) and PHF-1epitope tau phosphorylated levels for young and aged degus. A high correlation was observed for LTP and ORM. The values in parentheses indicate the number of animals used.

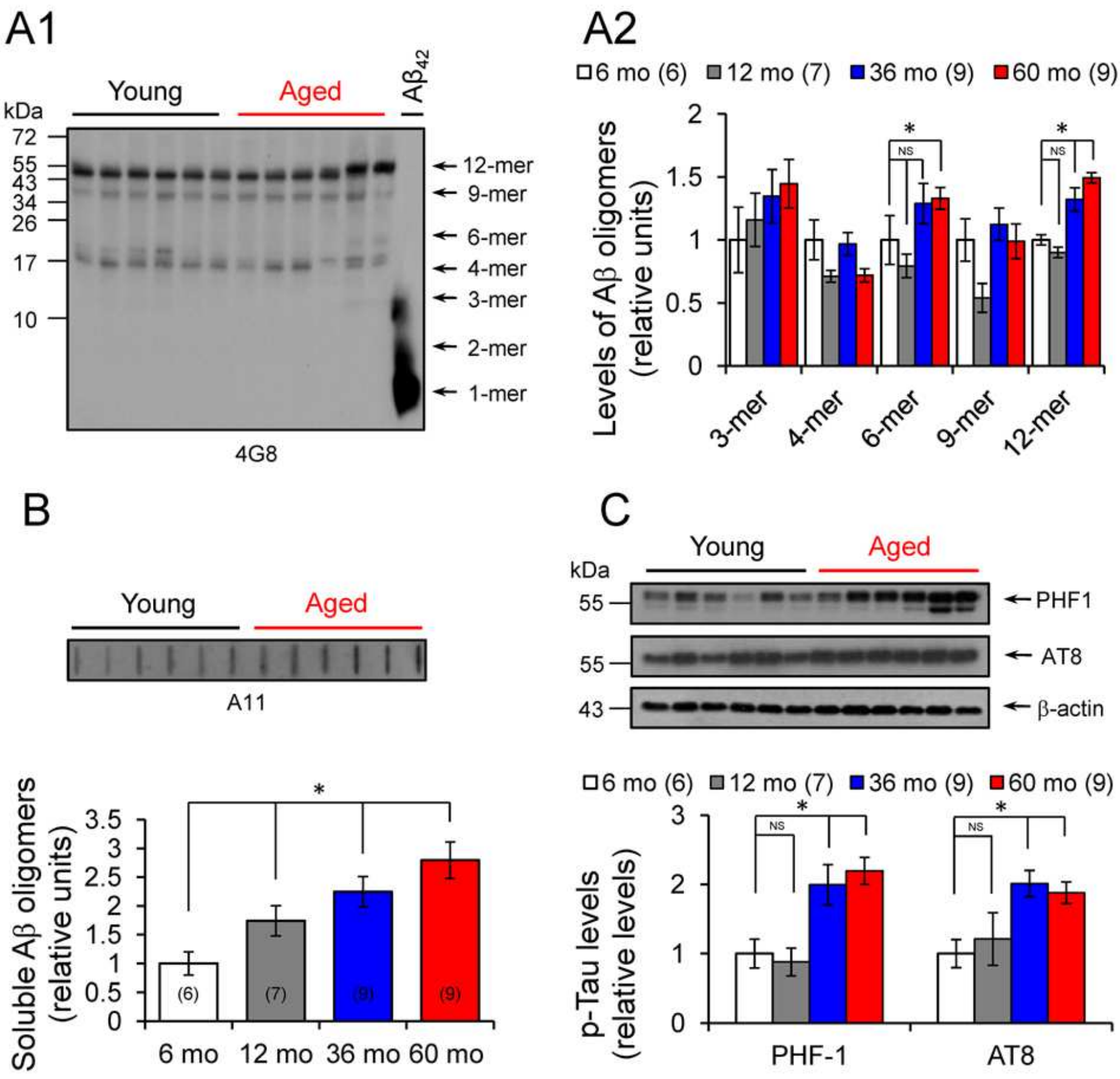
Fig. 7. Amyloid deposition and tau phosphorylation begins in the cerebral cortex of *O. degus*. A-D: Immunoreactivity for Aβ peptides using the specific antibody 6E10 showed extracellular (black arrows) staining in the hippocampus and the cerebral cortex from degus. A greater number of extracellular insoluble deposits were observed in aged (72 mo, C, D) compared to young (12 mo, A, B) degus. E-H: Immunodetection of pathological phosphorylated tau using the specific antibody AT8 in the hippocampus and cerebral cortex from degus. A greater number of positively stained somas (black arrows) were observed in aged (G, H) compared to young (E, F) degus. I-J: Quantification of Aβ burden (I, percentage of area occupied by 6E10-positive plaques) and the number of AT8-positive cells (J, number of neurons per area) for 6 mo (white, n=2), 12 mo (gray, n=2), 36 mo (blue, n=2) 60 mo (red, n=2) and 72 mo (black, n=2) revealed a significant increase after 36 mo in cortex and after 60 mo in hippocampus. One-way ANOVA ($F_{(4,88)} = 238.8$, *$p < 0.0001$ for 6E10-Hip), ($F_{(4,88)} = 646.4$, *$p < 0.0001$ for 6E10-Cx), ($F_{(4,88)} = 266.0$, *$p < 0.0001$ for AT8-Hip), and ($F_{(4,88)} = 134.9$, *$p < 0.0001$ for AT8-Cx) followed by Tukey´s post-hoc test ($p < 0.05$) compared to 6 mo.

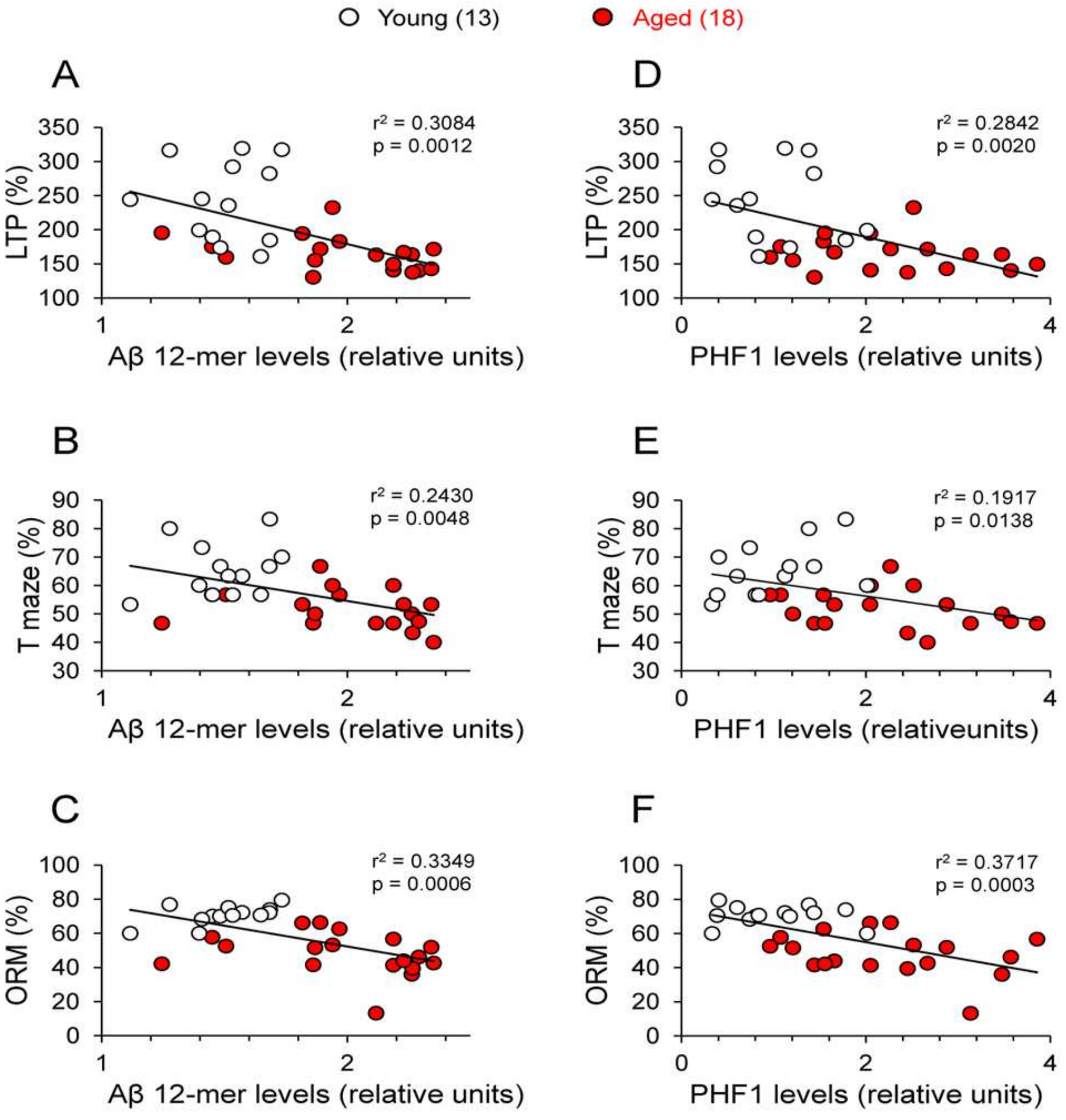

A
Young (13)
Aged (18)
N° of correct choices
Days
*
B
N° of correct choices
NS
*
(6)
(7)
(9)
(9)
6 mo
12 mo
36 mo
60 mo
C
Familiar
Preference index (%)
*
Young
Aged
D
Novel
Time with objects (s)
*
*
6 mo
12 mo
36 mo
60 mo

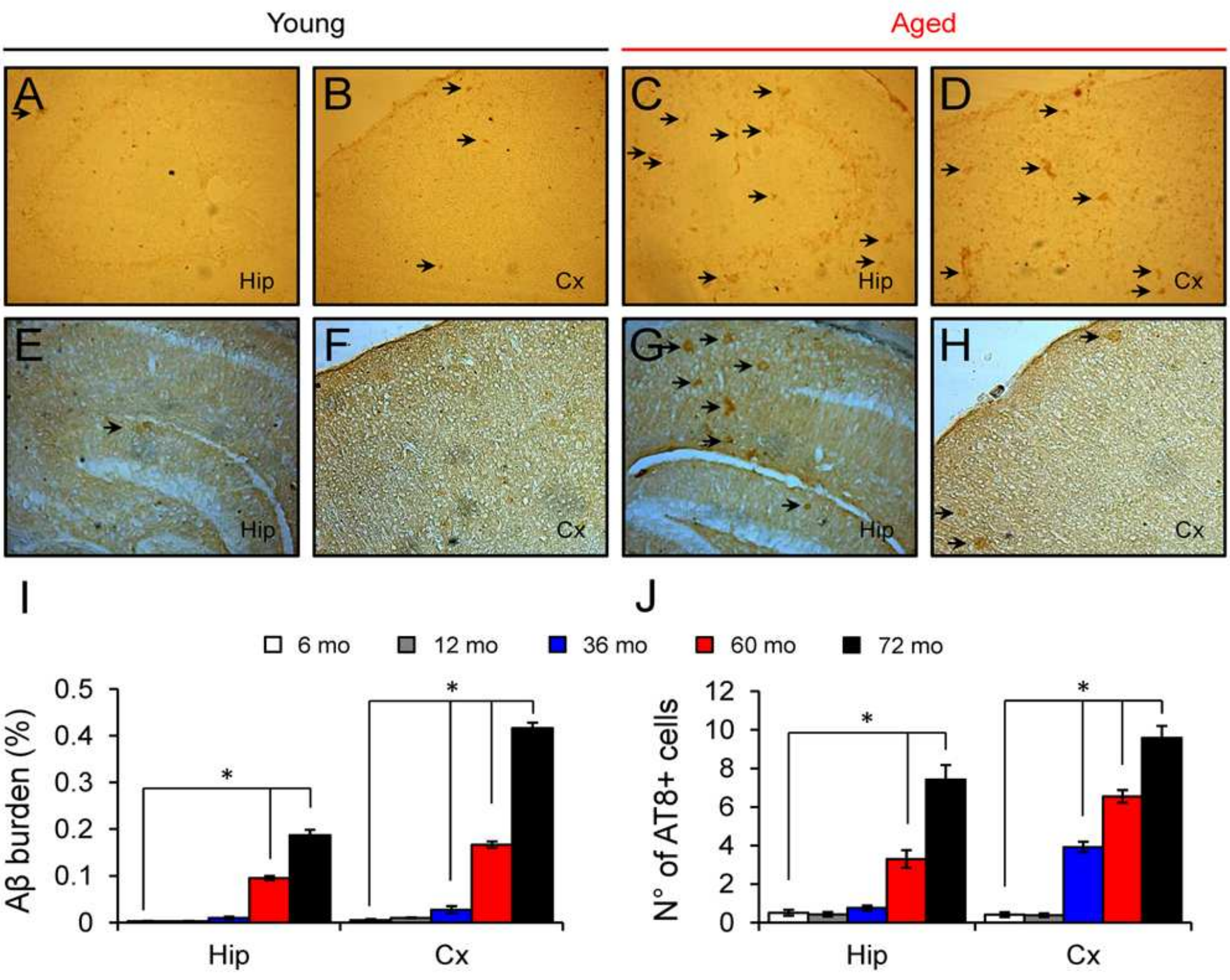

6 mo (12,5)
12 mo (13,5)
36 mo (20,6)
60 mo (17,5)
A1
fEPSP Slope (V/s)
FV amplitude (mV)
A2
FV amplitude (mV)
Stimulus intensity (μA)
A3
fEPSP slope (V/s)
Stimulus intensity (μA)
B1
Young (21,9)
Aged (29,14)
Facilitation ratio (R2/R1)
Interstimulus interval (ms)
B2
6 mo (10,4)
12 mo (11,5)
36 mo (15,8)
60 mo (14,6)
C1
mEPSC amplitude (pA)
mEPSC frequency (Hz)
Young
Aged
C2
cummulative probability
mEPSC amplitude (pA)
C3
Young (12,4)
Aged (9,3)

A
Young
Aged
kDa
43
95
130
170
55
SYP
PSD-95
GluR2
NR2b
β-tub
B
6 mo (6)
12 mo (7)
36 mo (9)
60 mo (9)
Synaptics proteins (relative units)
1.5
1
0.5
0
NS
NS
NS
*
*
*
SYP
PSD-95
GluR2
NR2B


A
Young (37,12)
1
2
Aged (85,17)
1
2
fEPSP slope (%)
400
300
200
100
0
-20
0
20
40
60
Time (min)
1
2
*
NS
*
(20,6)
(17,6)
(63,9)
(32,8)
6 mo
12 mo
36 mo
60 mo
B
Young (37,9)
2
1
Aged (29,11)
2
1
fEPSP slope (%)
100
50
0
-20
0
20
40
60
80
Time (min)
1
2
*
*
NS
(18,4)
(19,5)
(12,4)
(17,7)
6 mo
12 mo
36 mo
60 mo


A1
Young
Aged
I Aβ42
kDa
72
55
43
34
26
17
10
12-mer
9-mer
6-mer
4-mer
3-mer
2-mer
1-mer
4G8
A2
6 mo (6)
12 mo (7)
36 mo (9)
60 mo (9)
Levels of Aβ oligomers (relative units)
2
1.5
1
0.5
0
*
NS
3-mer
4-mer
6-mer
9-mer
12-mer
B
Young
Aged
A11
Soluble Aβ oligomers (relative units)
3.5
3
2.5
2
1.5
1
0.5
0
(6)
(7)
(9)
6 mo
12 mo
36 mo
60 mo
C
Young
Aged
kDa
55
PHF1
55
AT8
43
β-actin
6 mo (6)
12 mo (7)
36 mo (9)
60 mo (9)
p-Tau levels (relative levels)
3
2
1
0
NS
PHF-1
AT8


Young (13)
Aged (18)
A
LTP (%)
r² = 0.3084
p = 0.0012
Aβ 12-mer levels (relative units)
D
LTP (%)
r² = 0.2842
p = 0.0020
PHF1 levels (relative units)
B
T maze (%)
r² = 0.2430
p = 0.0048
Aβ 12-mer levels (relative units)
E
T maze (%)
r² = 0.1917
p = 0.0138
PHF1 levels (relativeunits)
C
ORM (%)
r² = 0.3349
p = 0.0006
Aβ 12-mer levels (relative units)
F
ORM (%)
r² = 0.3717
p = 0.0003
PHF1 levels (relative units)


Young
Aged
A
Hip
B
Cx
C
Hip
D
Cx
E
Hip
F
Cx
G
Hip
H
Cx
I
6 mo
12 mo
36 mo
60 mo
72 mo
Aβ burden (%)
0.5
0.4
0.3
0.2
0.1
0
*
*
Hip
Cx
J
N° of AT8+ cells
12
10
8
6
4
2
0
*
*
Hip
Cx