In Vivo Olfactory Model of APP-Induced Neurodegeneration Reveals a Reversible Cell-Autonomous Function

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Amyloid precursor protein (APP) has long been linked to the neurodegeneration of Alzheimer's disease (AD), but the associated cell death has been difficult to capture *in vivo*, and the role of APP in effecting neuron loss is still unclear. Olfactory dysfunction is an early symptom of AD with amyloid pathology in the olfactory epithelium correlating well to the brain pathology of AD patients. As olfactory sensory neurons (OSNs) regenerate continuously with immature and mature OSNs coexisting in the same olfactory epithelium, we sought to use this unique system to study APP-induced neurodegeneration. Here we have developed an olfactory-based transgenic mouse model that overexpresses humanized APP containing familial AD mutations (hAPP) in either mature or immature OSNs, and found that despite the absence of extracellular plaques a striking number of apoptotic neurons were detected by 3 weeks of age. Importantly, apoptosis was restricted to the specific population overexpressing hAPP, either mature or immature OSNs, sparing those without hAPP. Interestingly, we observed that this widespread neurodegeneration could be rapidly rescued by reducing hAPP expression levels in immature neurons. Together, these data argue that overexpressing hAPP alone could induce cell-autonomous apoptosis in both mature and immature neurons, challenging the notion that amyloid plaques are necessary for neurodegeneration. Furthermore, we show that hAPP-induced neurodegeneration is reversible, suggesting that AD-related neural loss could potentially be rescued. Thus, we propose that this unique *in vivo* model will not only help determine the mechanisms underlying AD-related neurodegeneration but also serve as a platform to test possible treatments.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder yet has no effective treatments. A pathological hallmark of AD is extracellular plaques containing amyloid- β (A β) peptides derived from amyloid precursor protein (APP). As genetic studies have also linked APP mutations to familial forms of AD, an amyloid hypothesis has been formulated that plaques cause the large-scale neuronal loss typical of late-stage AD patients (Hardy and Selkoe, 2002). While this provides a framework for AD-related studies, it remains controversial, as the density of amyloid deposits does not correlate with the degree of cognitive impairment observed in patients (Hardy and Selkoe, 2002). Transgenic studies overexpressing APP have also provided *in vivo* evidence that pathological and functional changes occur before plaque pathology, including inhibition of the ubiquit-

in-proteasome system, mitochondrial defects, synaptic dysfunction and cognitive deficits (Wirhns et al., 2004; Gouras et al., 2005; LaFerla et al., 2007; Bayer and Wirhns, 2010; Tappellini and Gouras, 2010). Interestingly, these pathologies have largely been attributed to intracellular A β accumulation, although the existence of low-level extracellular A β is difficult to completely rule out (Oddo et al., 2003). Unfortunately, most transgenic models poorly mimic the neurodegeneration of AD patients, such that APP mutations alone are sufficient to cause extensive neural loss in humans, but not in these models. For example, although APP23 mice show a modest loss of cortical and hippocampal neurons, it is far less than the loss observed in AD patients, while other models must involve additional disease-related proteins, and extensive aging, to produce neuron loss (Duyckaerts et al., 2008; Elder et al., 2010; Wirhns and Bayer, 2010). Thus, while APP has been implicated in effecting neurodegeneration, its role is poorly understood.

Olfactory dysfunction is one of the earliest symptoms of AD, often preceding cognitive deficits by many years (Bacon et al., 1998; Hawkes, 2003). Studies have shown that amyloid pathology in the olfactory epithelium (OE) parallels brain pathology (Arnold et al., 2010) along with neurodegeneration in the OE of AD patients (Talamo et al., 1989). We hypothesized that olfactory sensory neurons (OSNs) may be more sensitive to AD-related factors than cortical neurons and thus serve as a model to study APP-induced neurodegeneration. Since OSNs regenerate naturally from basal stem cells in the OE producing immature OSNs that migrate toward the apical surface as they mature (Farbman,

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Figure 1. Selectively overexpressing hAPP in either mature or immature OSNs. *a*, Schematic for generating transgenic lines that selectively overexpress hAPP in either mature (OMP promoter, *OMP-hAPP* line) or immature (*Gy8* promoter, *Gy8-hAPP* line) OSNs (see Materials and Methods). *b*, Colocalization of hAPP protein (red) with OMP protein (green) in *OMP-hAPP* line (left), and with GAP43 protein (green) in *Gy8-hAPP* line (right). Scale bar, 40 μm. *c*, Overexpression of human Aβ₄₂, Aβ₄₀ in *OMP-hAPP* line (Aβ₄₂, 0.016 ± 0.002; Aβ₄₀, 0.037 ± 0.005 μg/g; ratio, 0.44 ± 0.05; *n* = 5), and *Gy8-hAPP* line (Aβ₄₂, 0.015 ± 0.006; Aβ₄₀, 0.033 ± 0.010 μg/g; ratio, 0.46 ± 0.07; *n* = 5), compared with control, *tetO-hAPP* line (Aβ₄₂, 0; Aβ₄₀, 0 μg/g; *n* = 3). Values are mean ± SD. Animals: 3–4 weeks old.

1990; Calof et al., 1996; Cummings and Belluscio, 2008), the OE provides a continuous source of developing neurons. Here we have engineered two *in vivo* transgenic lines, which selectively overexpress a mutated humanized APP (hAPP) in either mature or immature OSNs, in a temporally controllable manner. We show that large-scale cell-autonomous neuronal death was readily and directly observed in OSNs, and could be rescued by turning off hAPP expression.

Materials and Methods

Transgenic lines. We used the Tetracycline-(tet-off)-transactivation system (tTA/tetO), allowing spatial-temporal control of transgene expression with the addition of doxycycline (Dox) turning-off promoter function (Gossen and Bujard, 1992; Lewandoski, 2001). *TetO-hAPP* line contains the hAPP transgene (humanized Aβ-domain with familial AD mutations—KM570, 571NL ‘Swedish’ and V617F ‘Indiana’) (Jankowsky et al., 2005). *OMP-tTA* and *Gy8-tTA* lines express the tetracycline transactivator in either mature or immature OSNs, respectively (Nguyen et al., 2007). Each tTA-containing line was crossed with *tetO-hAPP* line to generate lines that selectively express hAPP in either mature OSNs driven by the OMP promoter (*OMP-hAPP* line), or the immature OSNs driven by the *Gy8* promoter (*Gy8-hAPP* line). Presumably, hAPP expression begins during embryonic development when the OMP and *Gy8* promoters are activated (Nguyen et al., 2007). Genotyping was performed to recognize mutants containing both the tTA- and tetO-transgenes. Littermates containing only *tetO-hAPP* transgene were selected as controls and both sexes used for experiments. All mice were mixed (129 × C57BL/6) background.

PCR primers used for genotyping (forward, reverse): *OMP-tTA*, 5′-GGTTGCGTATTGGAAGATCAAGAGC-3′, 5′-GAGGAGCAGCTAG-AAGAATGTCCC 3′; *Gy8-tTA*, 5′-GTTCCAGCCCCAGTCCACACTCC-3′, 5′-CATGTCCAGATCGAAATCGTCTAGC-3′; *tetO-hAPP*, 5′-CCGAGATCTCTGAAGTGAAGATGGATG-3′, 5′-CCAAGCCTA-GACCACGAGAATGC-3′.

ELISA of Aβ. Colorimetric sandwich ELISA kits with antibodies against human Aβ₄₀ and Aβ₄₂ (Invitrogen) were used. Acutely dissected OE tissue was homogenized and centrifuged. Supernatant was loaded on ELISA plate. Assay was performed according to manufacturer’s manual with all standards and samples measured in duplicate.

Immunohistochemistry. Fluorescence immunohistochemistry on OE sections was performed as previously described (Cummings and Belluscio, 2010). Primary antibodies: APP, 1:1000; (6E10, Covance); OMP, 1:5000 (Wako); GAP43, 1:1000 (Novus Biologicals); cleaved-caspase3, 1:1000 (Cell Signaling Technology). Sections were examined using confocal microscopy (LSM510, Zeiss).

Immunoelectron microscopy. Previously established protocol (Tao-Cheng et al., 2006) was followed. *OMP-hAPP* mice were perfused transcardially with 2% PFA and 2% glutaraldehyde in 0.1 M PBS. Coronal sections of OE were incubated with an antibody against human APP (6E10, 1:11,000) followed by a Nanogold-conjugated secondary antibody (1:250; Nanoprobes). After dehydration, silver enhancement and staining with 2% uranyl acetate, tissue was embedded and sectioned, and examined with an electron microscope (1200EX II; JEOL).

BrdU pulse injection. BrdU (50 mg/kg body weight, Sigma) in 0.9% NaCl was intraperitoneal injected into 2-week-old mice. Later olfactory tissue was collected and sections were incubated in 2 N HCl at 37°C for 20 min to denature the DNA, before immunohistochemical staining using a BrdU antibody (1:500; Accurate Chemical).

Turning off hAPP expression. Doxycycline-containing chow (Dox-chow; 6 g/kg, 0.5 inch pellets, Bio-Serv) was fed to lactating mothers when *Gy8-hAPP* pups were 2 to 3 weeks old, and directly to *OMP-hAPP* mice from 3 to 4 weeks of age after weaning. Dox prevents the tTA protein from binding to the tetO-sequence.

Cell counting. OMP-, GAP43-, caspase3- or BrdU-positive cells in the septal OE spanning dorsal to ventral zones were counted manually using signal intensity and size threshold. Images of four to six sample sections were taken from each animal representing the anterior, middle, and posterior parts of the turbinates.

Statistical analysis. Student’s *t* test was performed to test statistical significance, assuming two-tailed distribution and two-sample unequal variance. All values are mean ± SD; **p* < 0.05 and ***p* < 0.001 in all figures.

Results

Selective overexpression of hAPP in two OSN populations

Studies have shown that specific molecular markers can be used to distinguish and manipulate mature versus immature OSNs: olfactory marker protein (OMP) for mature OSNs and G-protein gamma-subunit 8 (*Gy8*) or growth-associated protein 43 (GAP43) for immature OSNs (Verhaagen et al., 1989; Nguyen et al., 2007). Using these markers and the tetracycline-transactivation system we developed two *in vivo* transgenic lines that selectively express hAPP in either mature OSNs (*OMP-hAPP* line) or immature OSNs (*Gy8-hAPP* line) (Fig. 1*a*; Materials and Methods). This approach produced clear and specific hAPP overexpression in both mutant lines by 3 weeks of age that predominantly colocalized with OMP in the *OMP-hAPP* line and with GAP43 in the *Gy8-hAPP* line (Fig. 1*b*). Total APP protein in the mutants was ~2× endogenous levels (data

Figure 2. Rapid and widespread apoptosis in OSNs expressing hAPP. *a*, Mature OSN numbers (OMP-positive; green) were significantly smaller in both *OMP-hAPP* (230 ± 26 , $n = 5$) and *Gγ8-hAPP* (177 ± 41 , $n = 4$) than *tetO-hAPP* (505 ± 77 , $n = 6$), while immature OSNs (GAP43-positive; red) remained similar: *OMP-hAPP* (209 ± 42 , $n = 4$), *Gγ8-hAPP* (219 ± 31 , $n = 4$), *tetO-hAPP* (212 ± 31 , $n = 4$). *b*, Increased cleaved-caspase3 expression in mutant OE (white dots, collapsed confocal stack). *c*, Colocalization of cleaved-caspase3 (red) and hAPP (green) in both mature (*OMP-hAPP* line, top) and immature (*Gγ8-hAPP* line, bottom) OSNs (arrowheads). *d*, Significant increase in caspase3-positive OSNs in both *OMP-hAPP* (8 ± 2 , $n = 8$) and *Gγ8-hAPP* (9 ± 2 , $n = 4$), compared with *tetO-hAPP* (2 ± 1 , $n = 7$). However, there were fewer hAPP-expressing cells and a greater ratio of caspase3:hAPP-positive cells in *OMP-hAPP* (hAPP, 184 ± 58 ; ratio, $4.9 \pm 1.4\%$) than in *Gγ8-hAPP* (hAPP, 294 ± 62 ; ratio, $3.0 \pm 0.3\%$). Scale bars, $40 \mu\text{m}$. Values are mean $\pm$ SD per mm OE, single optical slice. Significance: $*p < 0.05$; $**p < 0.001$. Animals: 3–4 weeks old.

not shown). By comparison, transgene-derived $A\beta_{42}$ levels were $\sim 25\%$ of those reported in 4-week-old *CaMKII-hAPP* mice, which use a similar tTA-activation strategy, while $A\beta_{40}$ levels were similar (Jankowsky et al., 2005). Interestingly, both $A\beta_{42}$ and $A\beta_{40}$ levels, as well as the ratio between the two were comparable to those in the well noted “triple-transgenic” AD mouse at 5–7 months of age (Oddo et al., 2003) (Fig. 1c).

Rapid and widespread apoptosis in OSNs expressing hAPP

Immunohistochemical analysis revealed that in both of our mutant lines at 3 weeks of age, the OEs were thinner than in controls, and mature (OMP-positive) OSNs were significantly reduced while the immature (GAP43-positive) population did not change (Fig. 2a). Control mice had similar composition of mature and immature OSNs in the OE as previously reported (Vedin et al., 2009; Macdonald et al., 2010). To determine whether reduced mature-OSN population was due to elevated neuron death via apoptosis, as proposed by earlier studies in AD patients (Su et al., 1994; Troncoso et al., 1996), we performed cleaved-caspase3 immunohistochemical staining on OE sections. We found a striking increase in caspase3-positive cells in both mutant lines (Fig. 2b), which was approximately four times the control level (Fig. 2d). Importantly, caspase3 signal visibly colocalized with hAPP-expressing neurons (Fig. 2c) and did not occur in the neighboring population in the OE that did not express hAPP. For example, increased caspase3 signal was absent in the immature OSNs in the *OMP-hAPP* line, and absent in the mature OSNs in the *Gγ8-hAPP* line. Elevated apoptosis was further supported by TUNEL staining and condensed nuclear morphology that often colocalized with caspase3 signal (data not shown). Together, these data clearly demonstrated that hAPP overexpression alone was sufficient to induce neurodegeneration of OSNs through apoptosis. Notably, the ratio of caspase3-positive cells to hAPP-positive cells was greater in the *OMP-hAPP* line than in the *Gγ8-hAPP* line (Fig. 2d), suggesting that mature OSNs may be more vulnerable to hAPP-induced apoptosis than the immature ones.

Cell-autonomous hAPP-induced ultrastructural disruption of the OE

To determine whether hAPP-induced neurodegeneration was a consequence of extracellular plaques as proposed by the amyloid hypothesis, we examined the hAPP immunohistochemical signal in 3-week-old mutant mice using an antibody that recognizes both the full-length protein and the $A\beta$ peptide. Our data revealed clear intracellular signal but no evidence of aggregated extracellular signal (Fig. 3a). Using immuno-electron microscopy on *OMP-hAPP* OE, we further showed that hAPP labeling was predominantly confined to the nuclear-envelope, the lumen of the endoplasmic reticulum, and the plasma membrane facing the extracellular space, consistent with the intracellular trafficking route of APP (Thinakaran and Koo, 2008) (Fig. 3b). However, no aggregated extracellular gold particles were detected. We also observed broad ultrastructural changes in *OMP-hAPP* OE such as fewer dendritic knobs with fewer cilia, consistent with fewer mature OSNs (Reese, 1965; Schwob et al., 1992), and large numbers of apoptotic bodies (Fig. 3c–f).

Both mature and immature hAPP-expressing OSNs showed reduced survival but differential capacity for rescue

To determine at which stage of their life cycle OSNs died, we performed *in vivo* bromodeoxyuridine (BrdU) injections and collected OE tissue at different times postinjection. Earlier studies showed that immature OSNs reach maturity 6–7 d after their final division, thus defining a distinct transition point (Schwob et al., 1992; Carr and Farbman, 1993). In the *Gγ8-hAPP* line, most BrdU-labeled neurons disappeared before 7 d of age, consistent with the described immature time-window, whereas in the *OMP-hAPP* line, most BrdU-labeled neurons were lost between 7 and 14 d of age (Fig. 4a,b). These data indicated that mature and immature OSNs were both susceptible to hAPP-induced cell death. Interestingly, in the *Gγ8-hAPP* line, basal stem cell proliferation was upregulated, as indicated by twice as many BrdU-labeled cells 2 h postinjection compared with *OMP-hAPP* line and control, which had similar level of proliferation (Vedin et al., 2009; Macdonald et al., 2010). This difference could explain why the GAP43-positive population remained constant in *Gγ8-hAPP*

line despite the death of immature neurons and the depletion of mature OSNs (Fig. 2*a*).

Since expression of hAPP could be temporally controlled (see Materials and Methods), we next sought to determine whether the course of neuron loss could be reversed by shutting off the transgene. Mice were fed Dox-chow during the period when most BrdU-labeled cells disappeared in each mutant line (Fig. 4*c*). Dox treatment did not alter OSN survival in control mice (based on quantification of surviving BrdU-labeled cells, as well as caspase3- and OMP-positive cells in the OE, data not shown), yet successfully turned off hAPP expression in both mutant lines within 1 week (hAPP immunohistochemical signal was reduced to background level, data not shown). Our data showed that significantly more BrdU-labeled cells survived in the Dox-treated than in the untreated *Gγ8-hAPP* OE. There were also significantly fewer caspase3-positive cells and more OMP-positive cells in the Dox-treated *Gγ8-hAPP* OE. By contrast, Dox treatment in the *OMP-hAPP* OE showed only modest improvements in caspase3- and OMP-positive cells (Fig. 4*d*). Thus our data showed that hAPP-induced neurodegeneration could be reversed *in vivo*.

Discussion

This study presents a new *in vivo* model that allows direct and rapid visualization of hAPP-induced neuron death. Our data demonstrate that hAPP overexpression alone was sufficient to induce neuronal death, directly linking hAPP to the cause of neurodegeneration. Here we used an APP that contains two familial AD-mutations facilitating A β generation, but it will be interesting to determine whether both wild-type APP and other mutant forms resistant to secretase cleavage can also induce similar levels of apoptosis in OSNs. Since the cortical and hippocampal neural pathology in AD patients proceeds slowly, only a limited number of dying neurons can be detected at any one time, making it difficult to study the neurodegenerative process with most animals models (Yuan and Yankner, 2000). In this study, we show that in the olfactory system high levels of hAPP cause neurodegeneration via widespread apoptosis by 3 weeks of age, suggesting that susceptibility to hAPP-induced neurodegeneration might be linked to neuronal turnover. This may explain why regions that show high levels of neurogenesis, i.e., olfactory bulb (OB) and hippocampus, might also degenerate more rapidly (Hawkes, 2003; Takahashi et al., 2010). Interestingly, a recent study found that in mice overexpressing a mutated form of human-APP under the control of the prion-protein gene promoter (Tg2576 AD mice), the earliest amyloid deposits occur in the olfactory bulb, particularly the glomerular layer where OSNs project (Wesson et al., 2010). Since OSNs also express prion-protein, it is possible

Figure 3. Cell-autonomous hAPP-induced ultrastructural disruption of the OE. *a*, hAPP immunostaining showed intracellular cytoplasmic signal (arrowheads). *b*, Immunoelectron microscopic images of an OSN in OMP-hAPP OE. Black dots indicate hAPP signal (see Materials and Methods). Right panels correspond to boxed regions indicating hAPP protein in the nuclear (pink) envelope and ER (blue) lumen. *c*, *d*, EM images of control *tetO-hAPP* OE. *c*, Well-formed OSN dendritic knobs (left, arrowheads), each containing many cut cilia (right, arrows) and some intact (arrowheads). *d*, Spatially organized OSN cell bodies. *e*, *f*, EM images of OMP-hAPP OE. *e*, Very few smaller dendritic knobs (left, arrowhead) with less cilia (right, arrowhead). *f*, Disorganized OSN cell bodies around an apoptotic nucleus (red arrowhead). Scale bars: *a*, 10 μ m; *b*, 200 nm; *c*–*f*, 1 μ m. Animals: 3–4 weeks old.

that the OE of Tg2576 mice might also be degenerated thus contributing to the observed olfactory dysfunction. Similarly, we also noted extensive changes in the OB circuitry and impairment in odor detection in our system (data not shown).

Although both mature and immature OSNs were susceptible to hAPP-induced death, mature OSNs expressing hAPP had a higher percentage of apoptotic cells than immature OSNs. In addition, our data showed that hAPP-induced neurodegeneration could be rapidly reversed by shutting off hAPP expression in immature OSNs but more slowly in mature ones. The reason for this observed difference is not yet clear. Differential requirements for survival of mature versus immature OSNs, including neurotrophin dependence, neurotrophin receptor expression, odor-evoked activity, and turnover rate (Carson et al., 2005), could all potentially contribute to the observed difference. Likewise, extensive studies exploiting OB removal and lesion have shown that mature and immature OSNs have different vulnerability to target-dependent apoptosis (Schwob et al., 1992; Carson et al.,

Figure 4. Both mature and immature hAPP-expressing OSNs showed reduced survival but were differentially rescued by turning off hAPP transgene. **a**, Experimental timeline shows all BrdU injections in 2-week-old animals and olfactory tissue harvested 2 h, 7 d, or 14 d later. Images show OE immunostained for BrdU (white dots) corresponding to time points above. **b**, Quantification of BrdU-positive cells at 2 week (+2 h) point showed a significantly greater number in *Gγ8-hAPP* line (107 ± 18 , $n = 5$) than either *OMP-hAPP* (59 ± 6 , $n = 3$) or *tetO-hAPP* controls (52 ± 3 , $n = 4$) (black asterisk), revealing increased proliferation in *Gγ8-hAPP* line. A significant drop in BrdU-positive cells in 3-week-old *Gγ8-hAPP* mice (22 ± 7 , $n = 4$) compared with that in 2-week-old *Gγ8-hAPP* mice (red asterisks), as well as 3-week-old *OMP-hAPP* (69 ± 15 , $n = 4$) and *tetO-hAPP* (65 ± 21 , $n = 5$) (black asterisk) identified a susceptibility period of apoptosis in immature OSNs. In *OMP-hAPP* mice, a similar significant drop occurred between 3 and 4 weeks of age (4w: 9 ± 4 , $n = 4$; green asterisk), revealing a later susceptibility period in mature neurons. Both mutant lines had significantly fewer BrdU-positive neurons at 4 weeks of age (*Gγ8-hAPP*: 8 ± 1 , $n = 3$) than *tetO-hAPP* (43 ± 16 , $n = 4$) (black asterisk). **c**, Timeline of recovery experiments shows BrdU injections in 2-week-old mice, followed by Dox-chow (which turns off hAPP expression) given to *Gγ8-hAPP* mice from 2 to 3 weeks of age, and *OMP-hAPP* mice from 3 to 4 weeks of age (see Materials and Methods), based upon the susceptibility periods for each line determined above. **d**, OE from Dox-treated mice described in **c**, showing rapid recovery in *Gγ8-hAPP* line and delayed recovery in *OMP-hAPP* line. Graphs show significant changes in Dox-treated compared with nontreated *Gγ8-hAPP* OE, including increased BrdU-positive cells (Dox, 67 ± 14 , $n = 3$; no-Dox, 22 ± 7 , $n = 4$); decreased caspase3-positive cells (Dox, 4 ± 1 , $n = 3$; no-Dox, 9 ± 2 , $n = 4$) and increased OMP-positive cells (Dox, 383 ± 62 , $n = 3$; no-Dox, 177 ± 41 , $n = 4$). Dox-treated *OMP-hAPP* mice showed smaller changes compared with nontreated in BrdU-positive cells (Dox, 13 ± 5 , $n = 3$; no-Dox, 9 ± 4 , $n = 4$), caspase3-positive cells (Dox, 7 ± 3 , $n = 3$; no-Dox, 8 ± 2 , $n = 8$) and OMP-positive cells (Dox, 296 ± 22 , $n = 3$; no-Dox, 230 ± 26 , $n = 5$). Significance: * $p < 0.05$; ** $p < 0.001$. Scale bar, 50 μ m.

2005). Nevertheless, our BrdU experiments in immature OSNs show that initiating hAPP expression does not have to lead to cell death, suggesting it may be possible to rescue neurons undergoing hAPP-induced neurodegeneration. Moreover, early intervention may be a critical factor in determining recovery rate. Interestingly, previous studies have shown that reducing BACE1 levels can reduce degeneration of septohippocampal cholinergic neurons and memory deficits in 5XFAD mice, but the effect diminishes with age, also supporting the importance of early intervention (Devi and Ohno, 2010).

Together, our data argue that in OSNs hAPP-induced neuron death is through a cell-autonomous mechanism. First, extracellular plaques were not observed when widespread apoptosis was readily present in our model. Second, in both *OMP-hAPP* and *Gγ8-hAPP* lines, dying cells were confined to the OSN population that overexpressed hAPP, sparing the nonexpressing OSNs that reside side by side in the OE. Finally, neuron loss in this model could be rapidly reversed by turning off hAPP expression for merely a week, while extracellular amyloid deposits have been shown to be highly stable *in vivo* and can persist as long as 6 months after APP overexpression is turned off (Jankowski et al., 2005).

Earlier studies showed that in AD patients, there is a marked decrease in mature OSNs compared with the normal OE (Talamo et al., 1989). Deficits in odor detection and discrimination are among the earliest symptoms of AD and loss of mature OSNs could certainly contribute to the pathologies. Our model mimics mature-OSN reduction reported in AD-patients and suggests that the neurodegenerative process is similar to that in other brain regions. Since the OE is readily accessible for both biopsy and delivering biochemical compounds, we propose that this model will prove valuable not only for studying the pathological process of AD but also for developing diagnostics tools and determining the efficacy of potential therapeutics.

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