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BACE1 elevation is associated with aberrant limbic axonal sprouting in epileptic CD1 mice

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Abstract

The brain is capable of remarkable synaptic reorganization following stress and injury, often using the same molecular machinery that governs neurodevelopment. This form of plasticity is crucial for restoring and maintaining network function. However, neurodegeneration and subsequent reorganization can also play a role in disease pathogenesis, as is seen in temporal lobe epilepsy and Alzheimer's disease. β-Secretase-1 (BACE1) is a protease known for cleaving β-amyloid precursor protein into β-amyloid (Aβ), a major constituent in amyloid plaques. Emerging evidence suggests that BACE1 is also involved with synaptic plasticity and nerve regeneration. Here we examined whether BACE1 immunoreactivity (IR) was altered in pilocarpine-induced epileptic CD1 mice in a manner consistent with the synaptic reorganization seen during epileptogenesis. BACE1-IR increased in the CA3 mossy fiber field and dentate inner molecular layer in pilocarpine-induced epileptic mice, relative to controls (saline-treated mice and mice 24–48 h after pilocarpine-status), and paralleled aberrant expression of neuropeptide Y. Regionally increased BACE1-IR also occurred in neuropil in hippocampal area CA1 and in subregions of the amygdala and temporal cortex in epileptic mice, colocalizing with increased IR for growth associated protein 43 (GAP43) and polysialylated-neural cell adhesion molecule (PSA-NCAM), but reduced IR for microtubule-associated protein 2 (MAP2). These findings suggest that BACE1 is involved in aberrant limbic axonal sprouting in a model of temporal lobe epilepsy, warranting further investigation into the role of BACE1 in physiological vs. pathological neuronal plasticity.

Keywords

Aberrant neuroplasticity; Temporal lobe epilepsy; Mossy fiber sprouting; Dystrophic neurites; Beta-secretase; Alzheimer's disease

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Introduction

Synaptic plasticity is a fundamental property of the brain and is likely responsible for its ability to carry out many important biological functions, including cognitive activity (Luo and Yan, 2010). Synapses undergo constant plastic changes in response to internal and environmental stimuli (Zhang and Poo, 2010). This plasticity involves the removal and recreation of pre- and postsynaptic components in an activity-dependent manner, and can lead to neural network reorganization (Holtmaat and Svoboda, 2009).

Under some circumstances synaptic plasticity may go awry, resulting in the formation of aberrant brain circuitry and neural dysfunction. Drug addiction and obsessive–compulsive disorder have been deemed examples of such “negative synaptic plasticity” (Luscher and Malenka, 2011). In many neurological diseases, plasticity resulting from pathological damage to dendrites or axon terminals may contribute to, or exacerbate, disease progression. Aberrant axonal sprouting and swelling, as well as spine loss, have been observed in traumatic brain injury (Blizzard, et al., 2011; Dancause, et al., 2005; Marik, et al., 2010), cerebral stroke (Li and Carmichael, 2006), temporal lobe epilepsy (Ben-Ari, 2008; Jacobs, et al., 2000; Magloczky, 2010; Mikkonen, et al., 1998; Patrylo and Dudek, 1998; Sutula, et al., 1989) and Alzheimer’s disease (AD) (Akram, et al., 2008; Arendt, 2001; Cai, et al., 2010; Geddes and Cotman, 1991; Hashimoto and Masliah, 2003; Moolman, et al., 2004; Shim and Lubec, 2002; Stokin, et al., 2005).

The amyloidogenic proteins, including β-amyloid precursor protein (APP), β-secretase-1 (BACE1) and presenilins (PS1 and PS2) are well known to be responsible for amyloid pathogenesis in familial and sporadic AD (Hardy, 2009). However, these proteins also appear to play a key role in normal neuronal/synaptic development and plasticity (Laird, et al., 2005; Saura, et al., 2004; Seeger, et al., 2009; Wang, et al., 2008; Weyer, et al., 2011; Yan et al., 2004, 2007; Yu, et al., 2001). In addition, amyloidogenic proteins have been linked to neuronal responses to stress and trauma. APP overexpression/accumulation is widely documented in traumatic brain injury, stroke and epilepsy, and is often localized to dystrophic neurites (Gentleman, et al., 1993; Luan, et al., 2005; Nukina, et al., 1992; Sheng, et al., 1994). BACE1 upregulation has also been reported in the brain under various noxious conditions, including traumatic injury, ischemia and oxidative stress (Tong, et al., 2005; Velliquette, et al., 2005; Wen, et al., 2004; Xiong, et al., 2007).

Recent data suggest an association between epilepsy and AD. Specifically: 1) patients with AD have an increased susceptibility for unprovoked seizures (Amatniek, et al., 2006; Rao, et al., 2009; Shrimpton, et al., 2007); 2) amyloid plaques and tau pathologies have be identified in epileptic tissue (Gouras, et al., 1997; Mackenzie and Miller, 1994; Pollard, et al., 1994); and 3) murine models of AD often exhibit a pro-epileptic phenotype (Minkeviciene, et al., 2009; Palop, et al., 2007). However, it is not known whether amyloidogenic proteins play a specific role in epilepsy.

Systemic pilocarpine administration to rodents can induce acute status epilepticus and excitatory neuronal stress/injury, followed by synaptic reorganization and the development of spontaneous recurrent seizures (Curia, et al., 2008; Fabene, et al., 2007; Shibley and Smith, 2002; Tang and Loke, 2010). We have shown in transgenic models of AD that BACE1 upregulation is tightly associated with early-onset axonal swelling/sprouting (Cai, et al., 2012; Zhang, et al., 2009). Here we examined the spatiotemporal pattern of BACE1 elevation in brains of pilocarpine-induced epileptic CD1 mice to assess whether there could be a potential link between BACE1 overexpression and aberrant axonal sprouting in limbic structures in this experimental model of human temporal lobe epilepsy.

Materials and methods

Animals

Male CD1 mice (2–3 months old; Charles River; Portage, MI) were injected with methylscopolamine (1–1.2 mg/kg, i.p.) followed 15 min later by pilocarpine (290–295 mg/kg, i.p.) or vehicle (0.9% saline, i.p.). Animals were then closely monitored for 4–6 h to verify that they developed convulsive seizures (i.e., status epilepticus) sufficient to induce epileptogenesis and spontaneous recurrent seizures, i.e., at least 3 seizures with a seizure score ≥class III in severity over the next 2–3 h (Shibley and Smith, 2002). Approximately 50% mortality was observed among pilocarpine-treated animals. Surviving mice were returned to their home cages and were subsequently behaviorally monitored for 10–20 h per week to verify the onset of spontaneous recurrent convulsive seizures (i.e., an epileptic phenotype). A subpopulation of CD1 mice that exhibited seizures (i.e., status epilepticus) sufficient to induce epileptogenesis were sacrificed 24–48 h later to assess short-term histological alterations (n=3) for comparison with chronically epileptic animals (1–2 months later; n=7). Age-matched saline-treated controls (n=5) and pilocarpine-treated mice that did not exhibit an initial bout of seizures sufficient to induce epileptogenesis (i.e., 1–2 non-convulsive seizures over 6 h; n=7) were also allowed to survive for later comparative examination. Animal use was in accordance with the NIH Guide for the Care and Use of Laboratory Animals and all procedures were preapproved by the Animal Care and Use Committee at Southern Illinois University Carbondale.

Tissue preparation

For immunohistochemical studies, animals were deeply anesthetized with pentobarbital (100 mg/kg, i.p.) and transcardially perfused with phosphate buffer (PB, 0.1 M, pH=7.3) followed by 4% paraformaldehyde. Brains were removed, post-fixed overnight in perfusion fixative and then cryoprotected in 30% sucrose in phosphate-buffered saline (PBS, 0.01 M, pH=7.3). Each brain was then sectioned in the coronal plane using a cryostat, with sections from −2 to −4 mm caudal to bregma (Paxinos and Franklin, 2004) being collected. Twelve sets of 30 μm-thick sections were serially collected in cell culture wells, followed by 12 sets of 8 μm sections collected on positively charged microslides by thaw-mounting. The 30 μm-thick sections were used for avidin–biotin based immunohistochemistry and Nissl staining with cresyl violet for histological orientation, while the 8 μm sections were used for double immunofluorescence.

Immunohistochemistry

For avidin–biotin-based immunohistochemistry, sections were treated with 1% H_2O_2 in PBS for 30 min, pre-incubated in 5% normal goat serum with 0.1% Triton X-100 for 1 h, and then incubated with rabbit anti-neuropeptide Y (NPY) (1:4000, product #T4454, Peninsula Laboratories, Inc., San Carlos, CA) or rabbit anti-BACE1α (1:2000). For BACE1 labeling, antigen retrieval was used with sections treated in 50% formamide and 50% 2×SSC (0.15 M sodium chloride and 0.015 M sodium citrate) for 60 min at 65 °C (Zhang, et al., 2009). The sections were subsequently reacted with biotinylated goat anti-rabbit IgG at 1:400 for 1 h and then with freshly prepared avidin–biotin complex solution (ABC,1:400; Vector Laboratories, Burlingame, CA) for an additional hour. Immunoreactivity was visualized using a solution of 0.003% H_2O_2, 0.05% diaminobenzidine (DAB), 0.025% NiCl and 0.025% CoCl. Three 10-minute PBS washes were used between all incubations.

For double immunofluorescence, sections (8 μm, thaw-mounted onto slides) were preincubated in PBS containing 5% donkey serum for 30 min, and then in the same PBS buffer containing anti-BACE1α and one of the following antibodies: mouse anti-growth-associated protein 43 (GAP43) (1:4000, G9264, Sigma-Aldrich, St. Louis, MO); mouse anti-

polysialylated neural cell adhesion molecule (PSA-NCAM) (1:2000, MAB5324, Millipore); or mouse anti-microtubule associated protein-2 (MAP2) (1:1000, M9942, Sigma-Aldrich). Sections were then reacted for 2 h in Alexa Fluor® 488 conjugated donkey anti-mouse and Alexa Fluor® 594 conjugated donkey anti-rabbit IgGs (1:200, Invitrogen, Carlsbad, CA). Reacted sections were counter-stained with bisbenzimide (Hoechst 33342, 1:50000), washed 3 times in PBS and coverslippered with an anti-fading medium. The specificity of anti-BACE1α has been rigorously verified in several previous studies (Laird, et al., 2005; Xiong, et al., 2007; Zhang, et al., 2009). The specificity of other antibodies was tested by preabsorption of the primary antibody with neutralizing peptide and omission of the primary antibody in the immunolabeling protocol. Neither condition yielded specific immunolabeling in brain sections. For the purpose of densitometry, in all of our experiments brain-level matched control sections were processed along with the experimental sections, excluding the exposure to the primary antibody.

Imaging, data analysis and statistical testing

Sections were examined using an Olympus (BX60) fluorescent microscope equipped with a digital camera and an image analysis system (Optronics, Goleta, CA). Images (1600×1200 pixels) were taken at total magnifications of 40–400×. Optical density was measured in regions of interest using OptiQuant analysis software (Parkard Instruments, Meriden, CT) with specific optic density (o.d.) determined by subtracting the o.d. associated with non-specific immunoreactivity (assay control sections) from the total o.d. in experimental sections. Values of specific o.d.s were normalized relative to the means of saline-treated controls (100%) for graphical presentation. Data were statistically evaluated using Student's *t*-tests (two-tailed paired analysis) and one-way ANOVA with Bonferroni post-hoc tests (Prism GraphPad 4.1, San Diego, CA). Significance was set at $P < 0.05$.

Results

BACE1 elevation in sprouting mossy fiber terminals in epileptic mice

Given the heavy and distinct expression of BACE1 in the hippocampal mossy fiber pathway under normal conditions (Cai, et al., 2010; Laird, et al., 2005; Zhang, et al., 2009; Zhao, et al., 2007), we speculated that BACE1 immunolabeling would also be present in the aberrant mossy fiber sprouting seen in epileptic animals. Because NPY staining is associated with mossy fiber sprouting in epileptic animals (Borges, et al., 2003; Howell, et al., 2007; Nadler, et al., 2007), we spatiotemporally assessed BACE1 immunolabeling relative to NPY labeling in the hippocampal formation of epileptic relative to controls (Fig. 1).

NPY immunolabeling emerged along the mossy fiber terminal pathway in the hilus and CA3, but not the inner molecular layer, in pilocarpine-treated mice as early as 24–48 h after pilocarpine-induced status epilepticus (Figs. 1E, H). Virtually, no NPY labeling was observed in these locations in saline controls (Fig. 1D) or in pilocarpine-treated mice that did not undergo status epilepticus (not shown). NPY neoexpression appeared to increase in these regions at the longer survival times (i.e., the 1–2 month time point) (Fig. 1F) and also appeared in the aberrantly sprouted mossy fibers in the inner molecular layer of the dentate gyrus (Fig. 1I).

BACE1 immunolabeling in saline-treated controls and mice 24–48 h after pilocarpine induced status epilepticus was observed in the mossy fiber terminal zone in the hilus and CA3 (Figs. 1J, K, M, and N). A similar pattern of BACE1-IR was seen in pilocarpine treated mice that did not undergo status epilepticus (not shown). However, slightly increased BACE1-IR was observed in these locations in pilocarpine-induced epileptic mice (Figs. 1L,

O). Chronically epileptic mice also exhibited a clear band of BACE1 labeling in the inner molecular layer of the dentate gyrus (Figs. 1L, O; Figs. 2E, H).

Nissl staining was used to verify neuronal cell loss in pilocarpine-induced epileptic mice since this is a hallmark feature of temporal lobe epilepsy (Buckmaster and Dudek, 1997; Curia, et al., 2008; Tang and Loke, 2010). While no obvious cell loss was qualitatively observed in the hippocampal formation in mice 24–48 h after pilocarpine-induced status epilepticus (Fig. 1B), dramatic cell loss was evident in the hilus, CA1 and CA3 in the epileptic mice (Fig. 1C). Concurrently, the cross-sectional area of the hippocampal formation appeared to be reduced in the chronic epileptic groups relative to controls (Fig. 1A vs. C; D vs. F; J vs. L; Figs. 2A–C vs. E–G), suggestive of volume loss in the epileptic animals.

BACE1 elevation in additional temporal lobe regions in epileptic mice

In addition to being associated with mossy fiber sprouting, increased BACE1 immunolabeling was detected in CA1 and other limbic structures in epileptic mice relative to controls. This change in BACE1 expression was not noticeably accompanied by NPY neoexpression (Fig. 1F). In CA1, increased neuropil-like BACE1 reactivity was noted in epileptic mice relative to saline-treated controls, but not in mice that were sacrificed 24–48 h following pilocarpine-induced status (Fig. 1L, R vs. J, P and K, Q; Figs. 2E–H vs. A–D), or pilocarpine-treated animals that did not achieve status epilepticus (data not shown). At higher magnification, the enhanced CA1 neuropil BACE1 labeling was visualized in striatum oriens (s.o.) and stratum radiatum (s.r.), with numerous dot-like swollen profiles seen over these two layers as well as in stratum pyramidale (s.p.) (Fig. 1R; Fig. 2I). Similar to CA1, regionally increased neuropil-like BACE1 labeling was present in the amygdala as well as in the entorhinal and piriform cortices in epileptic mice (Figs. 2E–G, further illustrated in Figs. 3–5). This neuropil staining consisted of swollen profiles of small but varying sizes (Fig. 2K).

BACE1 elevation correlated with changes in axon terminals in epileptic mice

The pattern of increased BACE1 immunolabeling in epileptic mice appeared to be largely related to neuronal terminals as no perikaryal profiles were labeled in DAB-stained preparations. Therefore, we carried out double immunofluorescence and correlated densitometry to assess the localization of increased BACE1 labeling relative to markers of neuronal plasticity/growth. Quantitative analysis was obtained from batch-processed sections from epileptic mice using sections from the dorsal hippocampus at 3 levels (~500 μm apart), and data were normalized to age-matched saline-treated controls.

GAP43 plays an important role in axonal outgrowth and plasticity, and its distribution has been shown to be altered in the dentate gyrus of chronically epileptic rodents. Specifically, an increase in GAP43 occurs just above the granule cell layer or in the inner molecular layer, likely reflecting aberrant mossy fiber sprouting (Longo, et al., 2005; Tolner, et al., 2003). Synaptic reorganization in chronic temporal lobe epilepsy can also occur in CA1 (Esclapez, et al., 1999; Smith and Dudek, 2001) and extra-hippocampal areas (Ben-Ari, 2008; Jacobs, et al., 2000; Magloczky, 2010; Mikkonen, et al., 1998). We therefore compared BACE1/GAP43 labeling in multiple temporal lobe regions.

In the control mice, GAP3 IR was heavy in stratum lacunosum moleculare (s.l.m.) of CA1 and moderate in the molecular layer of the dentate gyrus, with only weak labeling in the mossy fiber terminals (Figs. 3A, C, and G). In the epileptic brain, a dramatic increase in GAP43 labeling was observed in stratum oriens (s.o.) and stratum radiatum (s.r.) of CA1 and CA3 as well as in the inner molecular layer in the dentate gyrus (Figs. 3D, F, and J).

This increased GAP43 labeling spatially correlated with the increased BACE1 immunolabeling at the same locations (Figs. 3D–F, J–L). In both CA1 and CA3, some swollen terminal-like profiles exhibited heavier BACE1/GAP43 immunofluorescence relative to the remaining local neuropil reactivity (Figs. 3F, J–L). Specific optical density (o.d.) verified that BACE1 and GAP43 were concurrently increased in CA1 in s.o. and s.r. (P=0.0001 and P<0.0001, respectively, Student's t-test) as well as in the dentate inner molecular layer (P=0.0005 and P=0.010; Fig. 3I). In s.l.m., GAP43 was comparable between epileptic and control mice (Fig. 3I). However, BACE1 density in this stratum was reduced in epileptic mice relative to controls (P<0.0001) (Figs. 3H, I, and K). As in CA1, BACE1 and GAP43 labeling was concurrently increased in the amygdala and piriform cortex of epileptic mice relative to age-matched saline-treated controls (data not shown).

PSA-NCAM is another molecule that is believed to be involved in neuronal plasticity and neuronal growth (Bonfanti, 2006). In saline-treated control mice, PSA-NCAM was expressed in the mossy fiber terminals at high levels and overlapped with strong BACE1 expression (Figs. 4D–F). Relative to controls, increased PSA-NCAM labeling was observed in s.o. and s.r. of CA3 and CA1 (Figs. 4H, J) as well as in the amygdala and piriform cortices (data not shown) in pilocarpine induced epileptic mice. The elevated PSA-NCAM labeling coexisted with increased BACE1 labeling in CA1 and CA3 (Figs. 4H, J–L). However, an increase in PSA-NCAM labeling was not observed in the inner molecular layer of the dentate gyrus (Figs. 4J, K). Densitometric analyses statistically confirmed significant increases in PSA-NCAM and BACE1 labeling in CA1 and CA3 of pilocarpine-induced epileptic mice relative to controls (Figs. 4M, N). In contrast, no difference existed between epileptics and controls in BACE1 or PSA-NCAM reactivity in the parietal cortex (Fig. 4O).

MAP2, a marker of dendritic profiles, generally exhibited an opposite trend of regional changes relative to BACE1 labeling in the epileptic mice (Fig. 5). Thus, in areas of CA1, amygdala, and piriform/entorhinal cortices where an increase in BACE1 labeling occurred, MAP2 immunoreactivity was reduced in epileptic mice (Figs. 5D–H) relative to control (Figs. 5A, B) or neighboring regions that did not exhibit increased BACE1-IR. Densitometric analyses confirmed a statistically significant reduction of MAP2 labeling and a statistically significant increase of BACE1 labeling in s.o. and s.r. of CA1, the inner molecular layer and hilus of the dentate gyrus (mossy fiber field) and the amygdala/piriform cortex (Fig. 5C; p values are indicated for individual regions, Student's t-test).

Discussion

Previous studies have reported elevation of BACE1 protein and activity following a variety of CNS insults (Tong, et al., 2005; Velliquette, et al., 2005; Wen, et al., 2004; Xiong, et al., 2007; Zhang, et al., 2010). In transgenic models of AD we have demonstrated an early-upregulation of BACE1 coinciding with axonal terminal sprouting and swelling, although this expression could result from primary or secondary effects of the transgenes (mutant APP or presenilin) (Cai, et al., 2012; Zhang, et al., 2009). Here we report clear anatomic evidence of increased BACE1 expression in the temporal limbic areas in epileptic rodents with an unmanipulated genetic background. The elevated BACE1 expression was not detectable at 24–48 h following the insult (i.e., status epilepticus), but was prominent in CD1 mice with chronic recurrent epilepsy. Overall, the increase in BACE1 appears to correlate with epilepsy-induced synaptic reorganization, as reflected by changes in NPY, GAP43 and PSA-NCAM. In comparison, MAP2, a marker for post-synaptic dendritic structure, negatively correlated with the change in BACE1.

BACE1 elevation marks progressive mossy fiber sprouting in epileptic CD1 mice

Several groups have noted strong and distinct BACE1 expression in the two most plastic synaptic relays in mammalian brain, namely the hippocampal mossy fiber terminals and the olfactory bulb glomeruli (Laird, et al., 2005; Yan, et al., 2007; Zhao, et al., 2007). BACE1 expression at these sites may be regulated by neuronal activity but is involved in modulation of synaptic transmission and long-term potentiation, consistent with a biological role of BACE1 in synaptic plasticity (Wang, et al., 2008; Zhang and Poo, 2010). Here we revealed an association of BACE1 upregulation with aberrant mossy fiber plasticity in a rodent model of temporal lobe epilepsy. By one month post-pilocarpine induced status epilepticus and afterward, increased BACE1 and NPY labeling in the dentate inner molecular layer became prominent, establishing the characteristic laminar pattern of aberrant mossy fiber sprouting in the epileptic hippocampal formation. Thus, it appears that BACE1 labeling can be used to assess the time course and pattern of mossy fiber sprouting in epileptic brain, complementing other known methods/markers such as Timm's stain, GAP43, NPY and zinc transport-3 (Chi, et al., 2008; Longo, et al., 2005; Nadler, et al., 2007; Tang and Loke, 2010; Tolner, et al., 2003).

BACE1 elevation reveals broad limbic axonal sprouting in epileptic CD1 mice

In human temporal lobe epilepsy, and in the pilocarpine rodent model of this disease, neuronal damage/death and synaptic/neuritic pathogenesis occur broadly in limbic areas. Both axonal sprouting and spine loss have been documented in the hippocampal formation, amygdala and piriform/entorhinal cortices in epileptic human or animal brain (Ben-Ari, 2008; Isokawa, 2000; Jacobs, et al., 2000; Kurz, et al., 2008; Mikkonen, et al., 1998; Tolner, et al., 2003). In the present study we found increased BACE1 labeling in hippocampal area CA1, amygdala and temporal cortices in epileptic mice up to 2 months after the of induction status epilepticus (in addition to the dentate gyrus and CA3 area). This increased BACE1 labeling appears to be largely localized to neuropil, with small granular and plexus-like profiles present in the same areas. The regionally increased BACE1 labeling was not associated with overt NPY neoexpression, probably due to the association of NPY with interneurons. Consistent with this notion, immunolabeling for GAP43 and PSA-NCAM, two common molecules linked to axonal sprouting or increased neuronal plasticity, was found to colocalize with the regional BACE1 increase, especially evident in CA1, CA3 and the amygdala/temporal cortex. As was noted above, a parallel increase in BACE 1 and PSA-NCAM staining was not observed in the inner molecular layer of the dentate gyrus. At present we cannot explain this discrepancy, but do not believe that it is due to inadequate sensitivity of our staining methods. In contrast to the generalized enhancement of PSA-NCAM and GAP-43 staining, immunolabeling of MAP2, a marker of dendrites and spines, was clearly reduced in the regions with increased BACE1 expression.

Factors/mechanisms potentially underlying BACE1 elevation in epileptic CD1 mice

Stress- or lesion-induced GAP43 and PSA-NCAM upregulation and MAP2 reduction have been documented in various traumatic paradigms, including seizures, which are often associated with neuronal injury/death and dendritic damage (Iijima, et al., 1998; Isokawa, 2000; Kurz, et al., 2008; Li, et al., 2000; Longo, et al., 2005; Pereno and Beltramino, 2010; Posmantur, et al., 1996; Taft, et al., 1992; Tolner, et al., 2003). Upregulation of growth-associated proteins under these conditions may be a molecular attempt at neuronal repair using enhanced neuroplasticity, including axonal sprouting (Arendt, 2001; Ben-Ari, 2008; Geddes and Cotman, 1991). BACE1 upregulation in sprouting limbic axon terminals seen in the brains of epileptic mice may be involved in synaptic regeneration in response to neuronal injury/death, and perhaps dendritic damage as well, given its anatomical association with axonal sprouting and biochemical correspondence with NPY, GAP43, PSA-NCAM neo/overexpression and MAP2 downregulation.

It is perhaps worth emphasizing that, while neuronal hyperactivity or hypermetabolism is recorded during an epileptic discharge, chronic temporal lobe epilepsy is characterized by cerebral hypometabolism during the interictal period, especially in the temporal cortex and hippocampal formation (Akman, et al., 2010; Henry, et al., 1993; Kuhl, et al., 1980). Such hypometabolism is also seen in the pilocarpine model of temporal lobe epilepsy (Dube, et al., 2001). We have previously shown an inverse correlation of BACE1 expression and oxidative metabolic activity (i.e., cytochrome oxidase reactivity) in the olfactory pathway (Yan, et al., 2007; Zhang, et al., 2010). Thus, it is reasonable to speculate that, among other previously mentioned factors, neuronal hypoactivity occurring due to neuronal damage, death and disconnection might be responsible for BACE1 upregulation in the epileptic limbic system.

BACE1 modulation of ion channel function

Emerging data suggest that BACE1 is also involved in cleavage of the beta (β) subunit of the sodium channel (Wong, et al., 2005). While the identity of all the different β and alpha (α) subunits of the sodium channel and their exact cellular localization in limbic areas is unclear, it is interesting to note that cleavage of the β subunit can affect overall channel function via controlling the localization, trafficking and inactivation of the pore-forming α subunit (Catterall, 2000; Isom, 2001; Schmidt and Catterall, 1986). Studies using BACE1 knock-out and transgenic mice suggest that surface expression of α subunits and neuronal activity can be either increased or decreased depending on the β and α subunits present/examined (Hu, et al., 2010; Huth, et al., 2011; Kim et al., 2007, 2011). Regarding epilepsy and seizures, BACE1 knockouts have been shown to exhibit spontaneous seizures (Kim, et al., 2007). In addition, changes in the β subunit of the sodium channel have been shown to be genetically linked with Dravet's syndrome (Patino, et al., 2009) and to affect the sensitivity to anti-epileptic drugs (Uebachs, et al., 2010). It is currently unknown whether an increase in BACE1 in the epileptic limbic system reflects either a compensatory response to epilepsy-induced hyperexcitability, or contributes to the hyperexcitability. While this will depend on the exact cellular localization of the specific α and β subunits, it is interesting to note that both a gain — and a loss — of sodium channel function have been observed with epilepsy (Claes, et al., 2001; Spampanato, et al., 2001). This is an area for future investigation.

Does BACE1 play an active role in axonal sprouting in the brain?

Our finding of BACE1 elevation in association with limbic axonal sprouting in epileptic CD1 mice raises at least two important questions. First, can this modulation lead to amyloid plaque formation? In the present study we failed to find extracellular Aβ deposition in areas with increased BACE1 labeling (data not shown), probably because wild-type rodents do not produce highly fibrillary Aβ species sufficient for amyloid aggregation and deposition. Other experimental models, such as transgenic AD mouse models, are better suited to examine this question. In any event, the current results suggest that BACE1 elevation in axonal components can occur in the absence of prior Aβ accumulation and deposition. A second issue concerns the biological role, if any, of BACE1 elevation in axonal sprouting and synaptic repair. In particular, does injury-induced BACE1 elevation serve as a physiological mechanism that either promotes or limits aberrant axonal growth and synaptogenesis? Interestingly, a recent study reported that reduced BACE1 activity enhanced the regeneration of axons in injured peripheral nervous system (Farah, et al., 2011). Further, an increase in BACE1 has been shown to affect beta subunit cleavage (β4) of the sodium channel, resulting in accelerated process outgrowth yet an overall decrease in overall number of filopodia-like extensions (Miyazaki, et al., 2007). It should be noted that BACE1 may also cleave other proteins important for cell secretion, adhesion, axonal growth and myelination, such as neuregulin and sialyltransferase (Kitazume et al., 2001;

Lichtenthaler et al., 2003; Farah et al., 2011; Luo et al., 2011). Given that BACE1 inhibition is being explored as a pharmacological treatment for AD or traumatic brain injury (Loane, et al., 2009; Luo and Yan, 2010; Mannix, et al., 2011), it will be critical to better understand whether, and if so, how, BACE1 plays an active role in synaptic remodeling and other neurobiological processes in the central nervous system.

In summary, BACE1 is a key enzyme for the genesis of Aβ, a major component of extracellular amyloid plaques. This enzyme is enriched at highly plastic synapses in the brain under physiological conditions, but is upregulated under stressful conditions. The present study demonstrates BACE1 overexpression in association with progressive mossy fiber sprouting in pilocarpine-treated epileptic CD1 mice. Moreover, BACE1 neoexpression occurred regionally in the hippocampus and limbic cortical areas following seizure induction, spatially colocalizing with increased GAP43 and PSANCAM immunolabeling but reduced MAP2 labeling. This pattern of results suggests a potential role of BACE1 in central axonal sprouting in the epileptic rodent brain.

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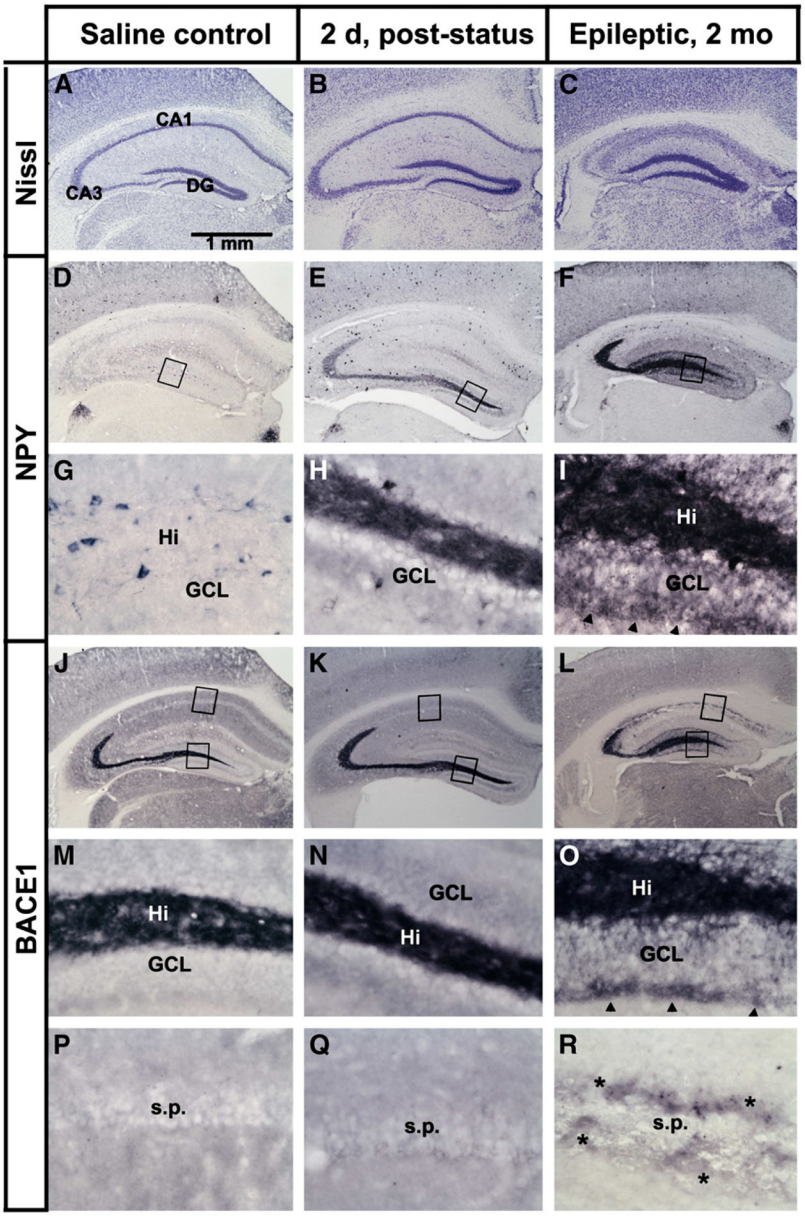

Fig. 1.

Immunolabeling for neuropeptide Y (NPY) and β-secretase-1 (BACE1) increased in the hippocampal formation of epileptic mice. Low and high magnifications mid-hippocampal sections are from a control animal (Saline control) and pilocarpine-treated mice that developed status epilepticus and were sacrificed after surviving 2 days (2 d, post-status) or 2 months (Epileptic, 2 mo). Cell loss in areas CA3 and CA1 was seen in epileptic mice that survived 2 mo (C) as compared to control (A) or 2 d survival (B). Compared to control (D, G), NPY neoexpression in the mossy fiber terminals emerges at 2 d (E, H) but intensified with longer survival time (F, I). Mossy fiber sprouting into the inner molecular layer (indicated by triangles in "I") was seen at the 2 mo survival time. NPY-labeled interneurons were seen in the cortex and hippocampal formation, including in the hilus (G). In control mice, BACE1 labeling in the control animal was prominent in the region of the mossy fiber pathway (J, M). The reactivity appeared to increase in the mossy fiber terminals from 2 d to

2 mo in the epileptic mice. Aberrant mossy fiber sprouting into the dentate inner molecular layer (indicated by triangles in “O”) was evident at the 2 mo survival point. Increased non-cellular labeling of BACE1 was also present in CA1 in 2 mo epileptic animals (L), with many darkly-labeled swollen terminals and processes present in the vicinity of the pyramidal cell layer (R, asterisks). DG: dentate gyrus; Hi:hilus; GCL: granule cell layer; s.p.: stratum pyramidale. Scale bar (in A)=1 mm for A–F and JL; bar=100 μm for G–I and M–R.

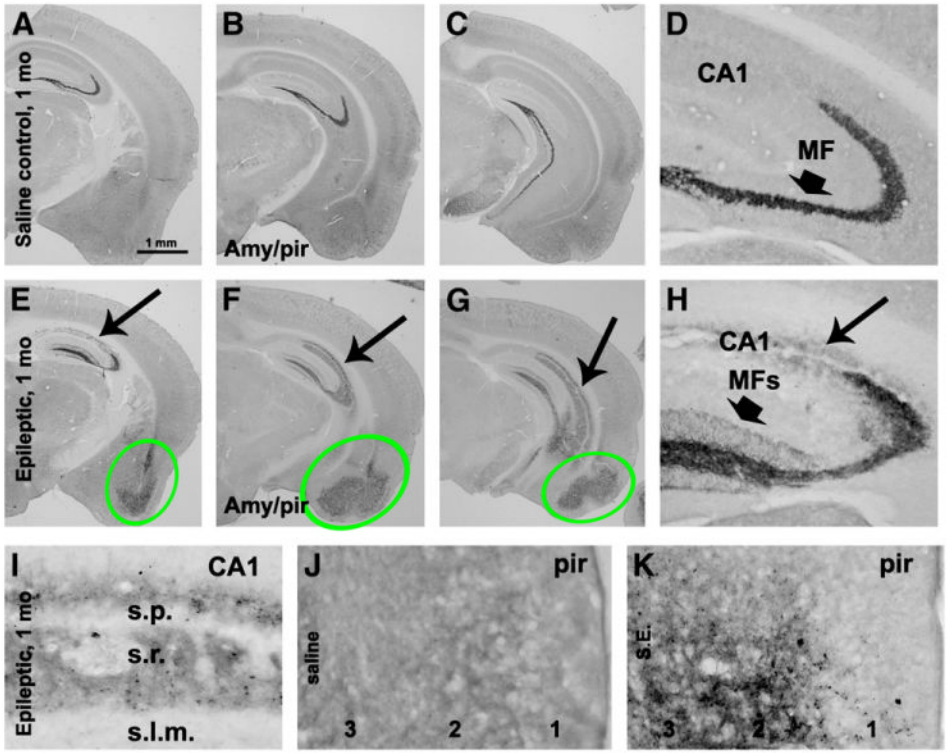

Fig. 2.

BACE1 immunolabeling in temporal lobe structures increased after one month of status epilepticus. Compared to control (A–D), neuropil-like BACE1 labeling in epileptic mice appeared regionally in the amygdala (Amy), piriform (pir) and entorhinal cortices (E–G), as indicated by the green circles. Mossy fiber sprouting (MFs) in CA3 and the dentate inner molecular layer was also observed in epileptic mice (H). In addition, a band-like labeling extended from CA3 into CA1 (indicated by large arrows in E–H) from stratum oriens (s.o.), through stratum radiatum (s.r.), but not including the pyramidal cell layer (s.p.) or stratum lucunosum-moleculare (s.l.m.) (I). Darkly labeled swollen puncta and processes were scattered in the hippocampus and piriform cortex (I, K). Arabic numbers indicate cortical layers (J, K). Scale bar (in A)=1 mm in A–C, E–G; bar=250 μm D, H and 100 μm for I–K.

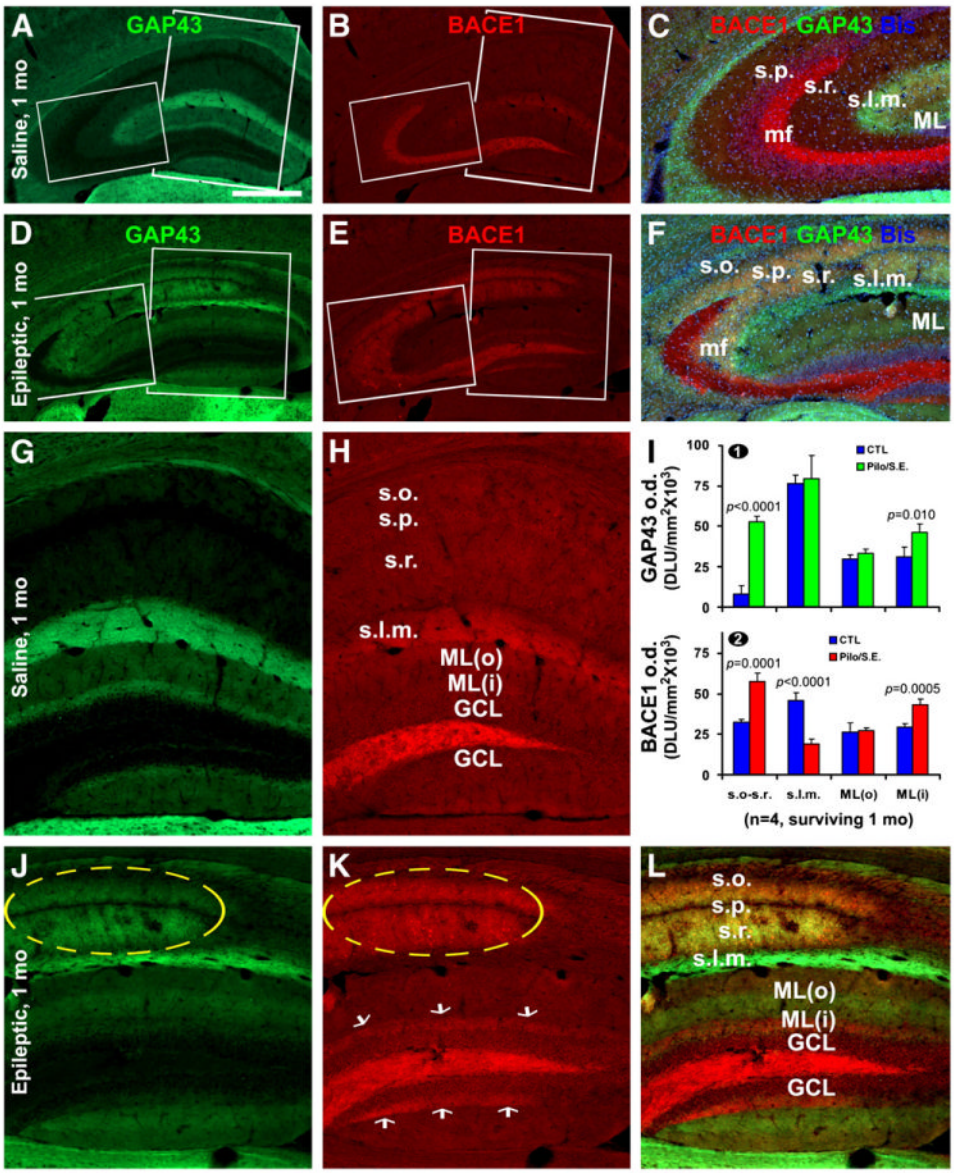

Fig. 3.

Double immunofluorescence showed a parallel increase of BACE1 and GAP43 labeling in CA1 and CA3 after one month of status epilepticus. Images A–C, G and H show normal distribution patterns of the two markers in the hippocampal formation, with framed areas enlarged/merged as other panels. In epileptic mice, increased GAP43 and BACE1 immunofluorescence co-occurred in CA3 and extended into the medial part of CA1 (D–F). Note overlap of GAP43 and BACE1 in CA1 shown by circled regions in J and K as well as in the merged image (L). Correlative densitometry revealed significantly increased specific optical density (o.d.) for both GAP43 and BACE1 in stratum oriens/stratum radiatum (s.o.–s.r.) in CA1 and the inner molecular layer [ML(i)] in the epileptics relative to controls (I). Specific o.d. of BACE1, but not GAP43, was reduced in the stratum lucunosum-moleculare (s.l.m.) (I). Bisbenzimide (Bis, blue) nuclear counterstain is included in merged images in C and F. White arrows in K highlight BACE1-positive mossy fiber sprouting. Other abbreviations — GCL: granule cell layer; s.p.=stratum pyramidale; ML(o): outer molecular layer. Scale bar (in A)=500 μm for A, B, D, E; bar=250 μm for the remaining images.

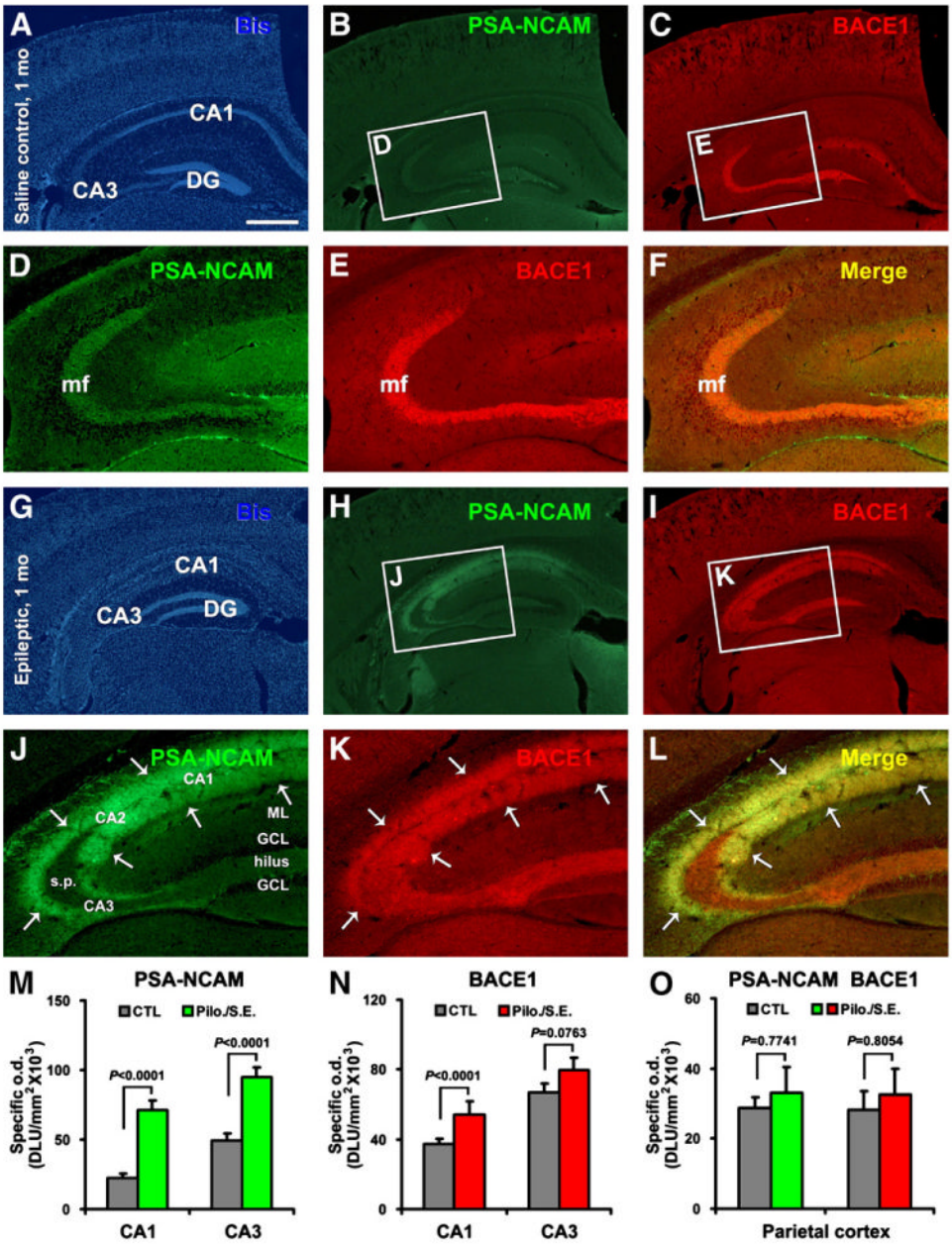

Fig. 4.

Double immunofluorescence showed a parallel increase of BACE1 and PSA-NCAM labeling in CA1 and CA3 after one month of status epilepticus. Images A and G are stained with bisbenzimide for orientation. Intense BACE1 and PSA-NCAM labeling is normally present in the mossy fiber terminal field (B–F). In the epileptic mice, increased labeling for both markers occurred in CA3 mossy fiber terminals (note particularly merged image in F) but also extended into CA1 (H–L). Bar graphs depict increased specific optical density (o.d.) of PSA-NCAM (M) and BACE1 (N) immunofluorescence in CA1 and CA3 in epileptic vs. control mice. No differences between groups for either marker were seen in parietal cortex (O). Scale bar (in A)=500 μm for A–C and G–I; bar=250 μm for the remaining images.

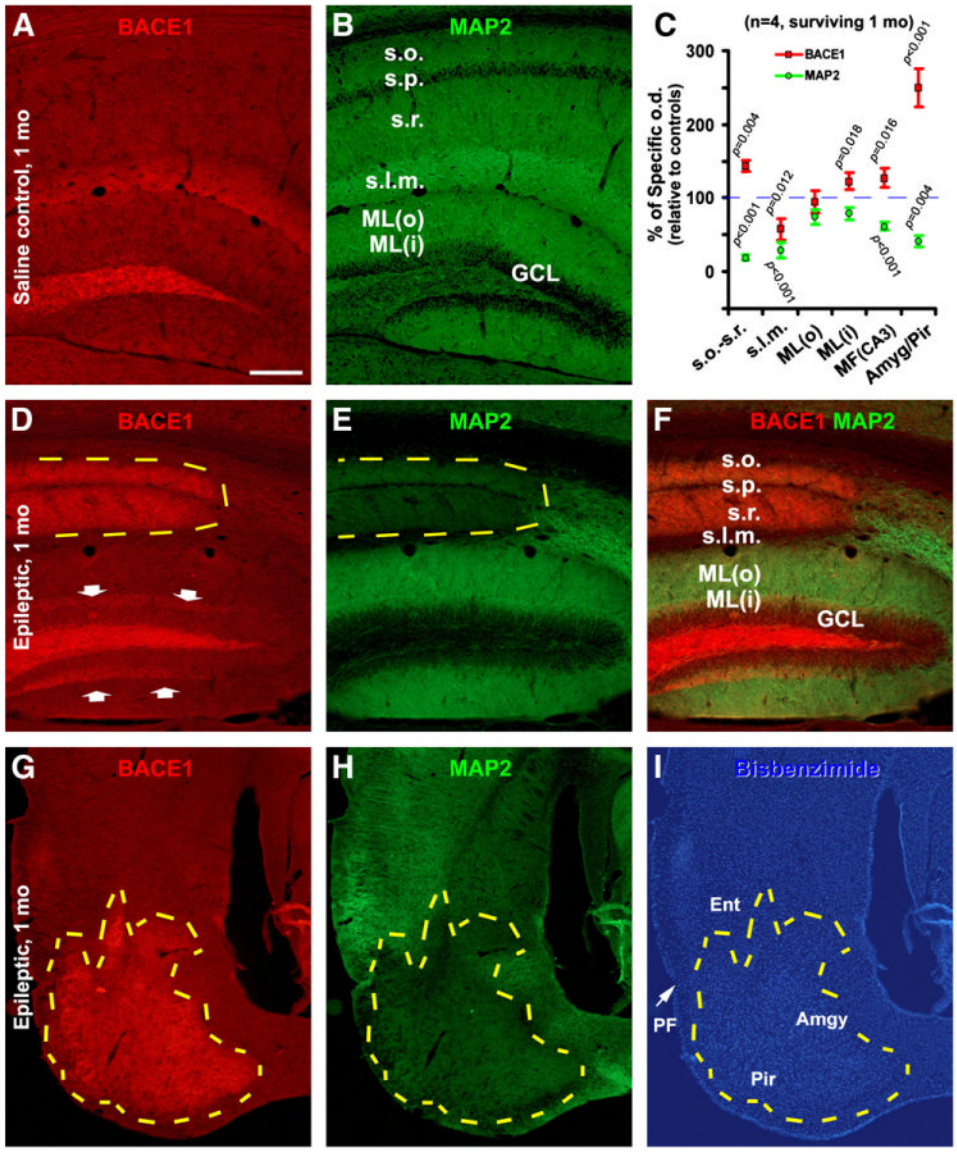

Fig. 5.

Opposing changes in regional BACE1 and MAP2 labeling in temporal lobe areas after one month of status epilepticus. Panels A and B show the normal pattern of BACE1 and MAP2 immunofluorescence in CA1 and the dentate gyrus from a control mouse. In the epileptic animals, BACE1 labeling increased but MAP1 labeling was reduced in area CA1 (D, E, outlined areas; merged in F) and the piriform/entorhinal cortex (G, H, outlined areas). Densitometry (C) confirmed increased BACE1 and reduced MAP labeling in CA1 in stratum oriens (s.o.), stratum radiatum (s.r.), dentate inner molecular layer [ML(i)], mossy fiber field (MF) in CA3 and the amygdala/piriform cortex in the epileptic mice. Of note, BACE1 and MAP2 densities were both reduced in the stratum lucunosum-moleculare (s.l.m.). Specific optical densities from the epileptic tissues are normalized to the means (i.e., defined as 100%, blue broken line in C) of control samples for corresponding measuring sites. PF: piriform fissure; Ent: entorhinal cortex; Pir: piriform cortex; Amyg: amygdala. Scale bar (in A)= 250 μm, and applies to all image panels.