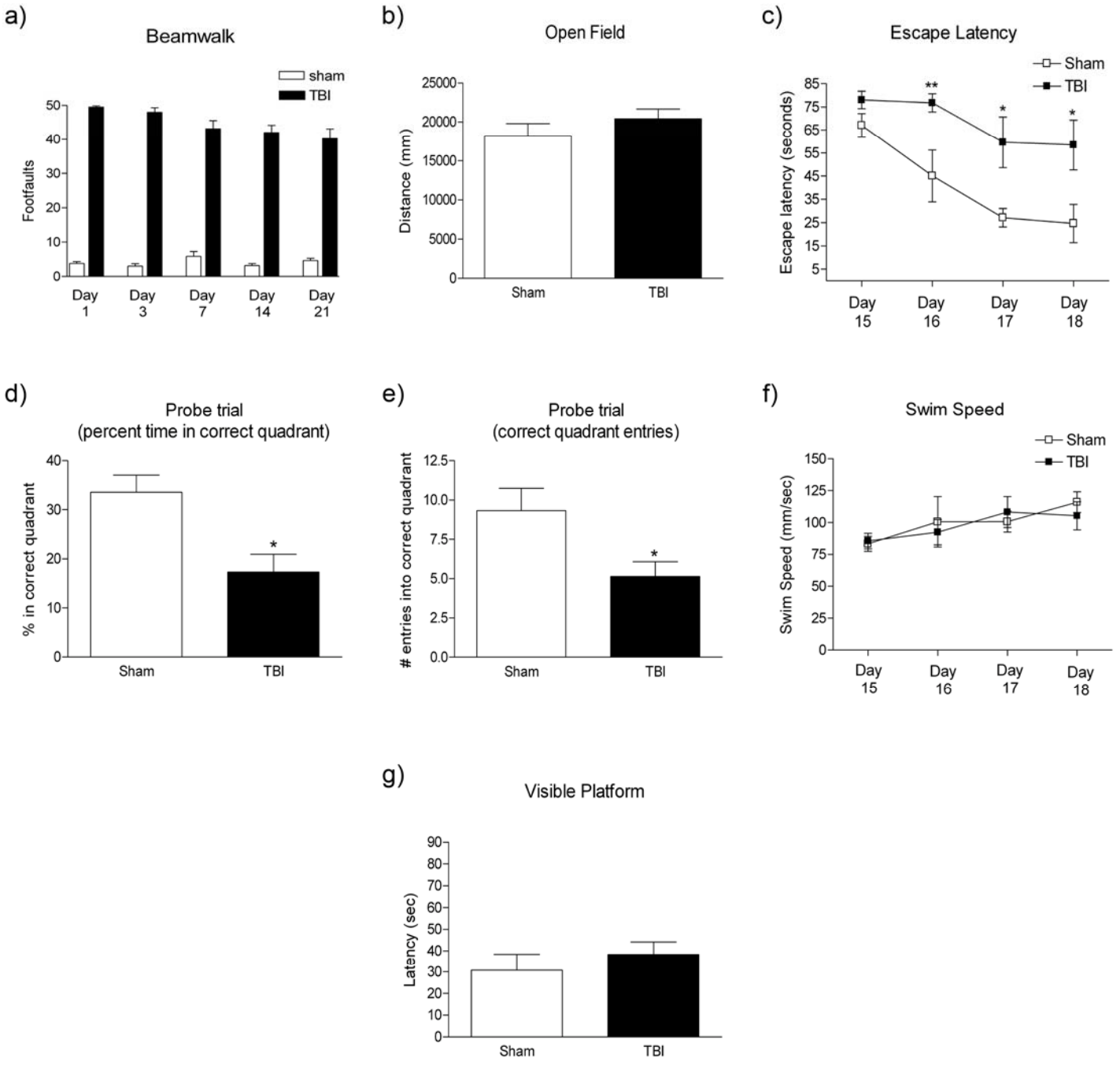
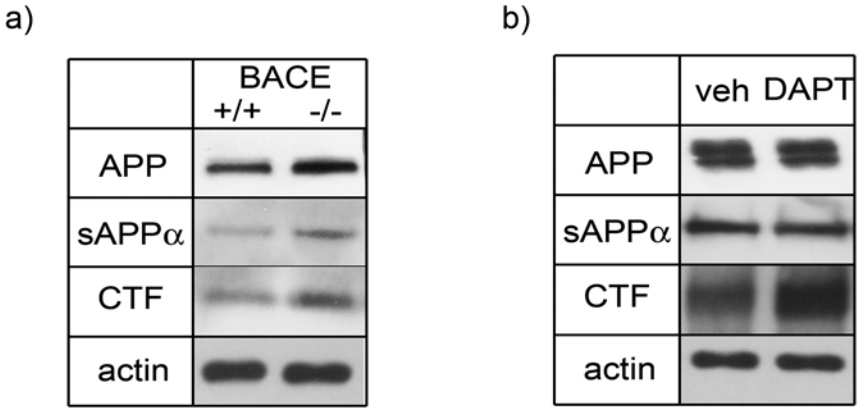


Supplemental Figure 1. A behavioral profile of the controlled cortical impact model of murine TBI (**a**) TBI causes deficits in fine motor coordination as measured by the beamwalk test. (**b**) These deficits in coordination are not caused by deficits in gross motor function, as ambulation in an open field paradigm remains unaltered. (**c**) Spatial learning was assessed in a Morris water maze on days 15-18, and escape latency was measured. TBI mice performed poorly, taking significantly more time to learn the position of the hidden platform (* = P<0.05, ** = P<0.01; ANOVA, Newman-Keuls post-hoc test, mean ± SEM. n = 8). Spatial memory deficits were assessed in a probe trial and % time in correct quadrant (**d**) and numbers of entries into correct quadrant (**e**) were measured. TBI mice were impaired compared to sham injured mice (* = P<0.05; Student t-test. mean ± SEM. n = 8). (**f**) The hippocampal impairments were not due to motor deficits as the swim speeds of TBI and sham injured animals were identical, and (**g**) similar times were recorded when a visible platform was placed in the maze.



Supplemental Figure 2. Although Aβ is the apparent target of the APP secretases, secreted APPα (a soluble fragment released following α-cleavage of APP) has been shown to be neuroprotective in a rat model of TBI (Thornton et al. (2006), Brain Res 1094:38). Previous studies have shown that BACE1 knockout mice have increased secreted APPα (Nishitomi et al. J Neurochem 99:1555), and we confirmed that finding in our mice (**a**). However, while this may be beneficial in BACE1-/- mice, this increase in sAPPα was not observed in DAPT treated mice (**b**).

(**a**) α-secretase cleavage is enhanced in BACE1-/- mice, with sAPPα and APP-CTF accumulating. (**b**) γ-secretase mediated cleavage is reduced in DAPT treated mice, with APP-CTF accumulating after 21 days of treatment.

Supplemental Methods

Animals

BACE1$^{-/-}$ and *BACE1*$^{+/+}$ mice were purchased from Jackson Laboratories. These mice were housed in Georgetown University Division of Comparative Medicine, and were 11-12 months old at the time of CCI. For the DAPT and timecourse studies, C57BL/6 mice were purchased from Jackson Laboratories and were 5-7 months old at the time of CCI. All procedures were reviewed and approved by the Georgetown University animal care and use committee.

Controlled Cortical Impact (CCI)

The CCI-injury device was designed and built at Georgetown University, and consists of a microprocessor-controlled pneumatic impactor with a 3.5 mm diameter tip[1]. Injury was induced by an impactor velocity of 6 m/s and deformation depth of 2 mm. Mice were anaesthetized with isoflurane (induction at 4% and maintenance at 1.5%) evaporated in a gas mixture containing 70% N_2O and 30% O_2 and administered through a nose mask. Depth of anesthesia was assessed by monitoring respiration rate and pedal withdrawal reflexes. The mouse was placed on a heated pad, and core body temperature was maintained at 37 °C. The head was mounted in a stereotaxic frame, and the surgical site was clipped and cleaned with Nolvasan scrubs. A 10-mm midline incision was made over the skull, the skin and fascia were reflected, and a 4-mm craniotomy was made on the central aspect of the left parietal bone. The impounder tip of the injury device was then extended to its full stroke distance (44 mm), positioned to the surface of the exposed dura, and reset to impact the cortical surface. After injury, the incision was closed with interrupted 6-0 silk sutures, anesthesia was terminated, and the animal was placed into a heated cage to maintain normal core temperature for 45 minutes postinjury. All animals were monitored carefully for at least 4 hours after surgery and then daily. Surgeries for individual studies were performed by the same model expert within a short timeframe (3 days) to minimize experimental variation, with control and treated groups randomly intermingled.

DAPT administration

N-[N-(3,5-Difluorophenacetyl-L-alanyl)]-sphenylglycinet-butyl ester (DAPT) was made up in 5% ethanol in corn oil and was administered at a volume of 5ml/kg and a final dose of 30mg/kg. Vehicle was 5% ethanol in corn oil, and was administered orally at a volume of 5ml/kg. Each mouse received the initial dose 15 minutes after CCI or sham surgery, and twice daily for 21 days.

Behavioral Testing

Beamwalk: Fine motor coordination was evaluated for all animals using a beam walking task, a method which is particularly good at discriminating coordination differences between injured and sham-operated animals[1]. The device consists of a narrow wooden beam 6 mm wide and 120 mm in length, which is suspended 300 mm above a 60 mm thick foam rubber pad. The mouse was placed on one end of the beam and the number of footfaults for the right hindlimb recorded over 50 steps counted in either direction on the beam. A basal level of competence at this task was established before surgery with an acceptance level of <10 faults per 50 steps. The beam walk task was performed at 1, 3, 7, 14 and 21 days after injury.

Open Field: Gross motor ability was measured using a novel open field paradigm. Briefly, mice were placed in a novel, brightly lit, circular arena (3ft x 3ft). A TOPSCAN tracking system (Clever Sys Inc.) was used to quantify ambulation distance over a 5 minute trial period.

Morris Water Maze: A Morris Water Maze paradigm was employed to assess spatial learning by training mice to locate a hidden, submerged platform using extramaze visual information, as previously detailed[1]. The apparatus consists of a large, white circular pool with a plexiglass platform painted white and submerged below the surface of the water, which is rendered opaque with the addition of a white non-toxic paint. During training, the platform was hidden in one quadrant of the maze 14 inches from the side-wall. The animal was gently placed into the water facing the wall at one of four randomly chosen quadrants separated by 90 degrees. The time required (latency) to find the hidden platform with a 90 second limit was recorded by a blinded observer and tracked using TOPSCAN. Animals failing to find the platform within 90 seconds were assisted to the platform. Animals were allowed to remain on the platform for 15 seconds on the first trial and 10 seconds on all subsequent trials. A series of trials administered in blocks of four were conducted on days 15, 16, 17 and 18 post-injury. A probe trial of 90s

was given 24 hours after the final learning trial. The percentage of time spent in the quadrant where the platform was previously located was recorded. To control for visual discriminative ability or motor impairment, the same animals were finally required to locate a clearly visible black platform (placed in a different location) raised 5 mm above the water surface at least 2 hours after the last trial. It is important to note that although significant differences can be observed on the first day of testing, this is due to the average latency of four trials being recorded on that day. On the first test (of four) in the first trial, no significant differences were observed between groups, with all animals failing to locate the platform.

MRI measurement

T2-weighted magnetic resonance imaging (MRI) at 21 days was used to visualize lesion following TBI, as previously detailed[2]. At 21 days after TBI all animals were anesthetized using isoflurane (induction at 4% and maintenance at 1.5%) evaporated in a gas mixture containing 30% oxygen/70% nitrous oxide and applied through a nose-mask, and subjected to MRI using a Bruker 7T/21 cm Biospec-Avance system. Briefly, animals were placed within a plexiglass animal bed with a heating pad warmed to 37 °C to maintain the animal's temperature. The animal bed was positioned so the animal's head was in the center of the magnet within a 72 mm ^{1}H birdcage resonator. Field homogeneity across the brain was optimized and a sagittal scout image acquired (RARE image, FOV=4×4 cm, 128×128 resolution, TR/TE=1500/10 ms with a rare factor of 8 making the effective TE=40 ms). Multi-slice T2-weighted images were then acquired to obtain sixteen contiguous slices commencing at the end of the olfactory bulb and working caudally (FOV=3×3 cm, slice thickness=0.5 mm, 256x256 resolution, TR/TE=1500/20 ms, four echo images, and four averages).

Histology

Mice were anesthetized and transcardially perfused with saline and 10% paraformaldehyde. The brains were removed, stored in fresh 10% paraformaldehyde overnight, protected in 30% sucrose, frozen in O.C.T. media, sectioned (20 m), and mounted onto slides. Sections were dried overnight at room temperature, and stained with Gill's hematoxylin, followed by counterstaining in 2.5% eosin and coverslipped. Lesion volume was assessed based on the Cavalieri method of stereology using Stereologer software (Systems Planning and Analysis). Every fifth brain section from 5 mm rostral to 5 mm caudal the lesion site was probed. In addition, sections were randomly selected for neuronal staining and cell counts. Briefly, sections were immunostained with anti-NeuN (Chemicon) for 1 hour, washed in PBS and incubated with biotinylated anti-mouse IgG antibody (Vector Laboratories) for 1 hour at room temperature. Sections were placed in avidin–biotin–horseradish peroxidase solution, diluted according to the manufacturer's instructions for 30 minutes (Vectastain elite ABC kit) and then reacted with 3,3′-diaminobenzidine (Vector Laboratories) and H_2O_2 for colour development. Positively stained neurons were viewed by light microscopy. Total neuronal cell counts in hippocampal CA1 subfields were obtained by a blinded investigator using unbiased stereology analysis (Stereologer). Every fifth brain section from 5 mm rostral to 5 mm caudal the lesion site was analyzed beginning from a random starting point. The multi-level sampling design in the Stereologer software, based on the optical fractionator sampling method, was used to estimate DAB-stained neuronal cell numbers. Neurons that fell within the counting frame or were touching the inclusion lines were counted as the nuclei came into view while focusing through the *z*-axis.

Aβ ELISA and Western blotting

Proteins for ELISA and western blot were sequentially extracted from isolated cortex in diethylamine and RIPA buffer as previously described[3]. Endogenous mouse $A\beta_{x\text{-}40}$ was detected from diethylamine (DEA) extracts using a commercially available kit from Wako Chemicals (Richmond, VA), as per manufacturers instructions. Western blotting was performed as previously described[4]. DEA extracts were probed for sAPPα (clone 2B3, IBL). RIPA extracts were probed with antibodies against full length APP (clone 22C11), APP-CTF (clone C1/6.1, gift from Dr. Paul Mathews), *BACE1* (Chemicon), PS-1 (Chemicon) and β-actin (Sigma-Aldrich).

Supplemental References

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