Supporting Information

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Case History

E.P. was a right-handed, white male born in 1922. He grew up in Castro Valley, a central California agricultural community. He obtained 12 y of education and then traveled at sea as a radio operator for an oil company (1941–1950). He then moved to Los Angeles County where he worked for 28 y as a technician in the aircraft industry and for 5 additional years as a part-time consultant. He moved to San Diego County in 1993. He was married (since 1950) and had two children.

In November 1992, at the age of 70 y, E.P. became ill with flu-like symptoms (fever and lethargy) and an episode of memory loss. His memory then worsened during the subsequent few days, and he was admitted to the hospital where he was diagnosed with viral encephalitis and given a 10-d course of i.v. acyclovir. T2-weighted MRI images of the brain acquired 2 wk after admission revealed abnormal signal enhancement compatible with diffuse, bilateral, and symmetrical encephalitis. In early 1993, he was reported to be unable to identify any smells in either nostril. By June 1993 his condition had stabilized, but profound memory impairment persisted for the remainder of his life.

E.P. was an affable man, always agreeable and cooperative during testing sessions. During sessions involving computer-administered tests, he would remark repeatedly about the invention of portable electronics and how in his day "this [laptop] would be in a 6-foot rack." Within a 1-h testing session, E.P. could make the same comment as many as 10 times. His conversations with research staff were limited to comments about the testing session and stories about his early life, e.g., his childhood on a farm, his teenage interest in ham radio, and his job as a radio operator on an oil tanker. He did not speak about more recent events. At the age of 84 y, he once was asked "How old are you now?" He replied, "Let's see, 59 or 60. You got me. My memory is not that perfect." He did not have awareness of a serious memory problem, because his memory impairment after his illness was so severe that he never acquired a different view of himself.

He lived a sedentary life. On typical days he had breakfast upon awakening and then returned to bed to listen to the radio. When arising again, he often had breakfast again. His wife reported that he would sometimes have breakfast three times before staying up for the day. He needed no assistance in carrying out most activities of daily living, such as bathing or shaving, although his wife reported that he often would need reminders. During the day, he would take short, L-shaped walks on the sidewalk around his corner house or sit in the backyard or living room. He also watched television and read the newspaper. His wife reported that he sometimes would suggest that they go out (e.g., to go shopping), but once they left he would become confused and ask to return home.

E.P. had a history of hypertension and arthritis, hernia repair, and an uncomplicated myocardial infarction with complete recovery and no indication of cerebral ischemia. He stopped smoking in 1959 and, according to his wife, was never a heavy drinker. He had no history of heart or lung disease, seizures, or loss of consciousness. A neurological examination in 1995 found E.P. to be alert and attentive but disoriented to place and time (month and year). His language was fluent. He could copy geometric figures and had no signs of spatial neglect. Examination of cranial nerves revealed anosmia. Reflexes, posture, motion, and somatosensory function were normal.

E.P. participated in our studies from 1994 to 2008, and his testing contributed to 35 publications. He showed no signs of cognitive decline during this period. Research staff visited him more than 200 times, although they never came to be recognized as familiar. On January 28, 2008, E.P. was hospitalized with a broken hip. He had successful hip replacement surgery and in February was transferred to a nursing facility near his home for rehabilitation. There he suffered a cardiac arrest and died on March 3, 2008.

SI Materials and Methods

Preparation of Brain for Sectioning and Histological Processing. Before the brain was frozen for sectioning, it was photographed from multiple orientations. The cerebrum then was embedded in a cylindrical cast of 10% gelatin that was hardened in a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 7 d. The embedded brain then was cut coronally into three thick tissue blocks. A custom-made slicing apparatus ensured that the resulting faces of the blocks were parallel to each other and that there was minimal tissue loss at the cut interfaces. The anterior cut was made just in front of the temporal pole. The posterior cut was made at the level of the splenium of the corpus callosum. Given this blocking scheme, the middle tissue block included the temporal lobes and all subcortical structures (with the exception of the pulvinar). This block included the entire span of the lesion that was visible on the surface of the brain. The brainstem and cerebellum were dissected at the level of the midbrain.

The three blocks of tissue were cryoprotected in increasingly concentrated phosphate-buffered solutions of sucrose (10–30% by volume). This process took ~16 wk to complete. After the blocks were completely infiltrated, they were frozen rapidly in a chilled bath of isopentane (−40 °C) essentially according to the method of Rosene et al. (1).Whole coronal sections were cut at a thickness of 70 μm using a custom-engineered, large-format sliding microtome (modified from a Reichert-Jung Tetrander Sliding Microtome, Model 29007). Tissue sections were stored in sequential order at 4 °C in 0.1 M phosphate buffer and 0.01% sodium azide. One in every 24 sections was mounted on 5 × 7 inch glass slides, dried at room temperature for 48 h, and stained for Nissl substance using a 0.2% solution of thionin in acetate buffer. Briefly, batches of 50 sections were defatted in 100% alcohol/chloroform and then were rehydrated in a progressive series of decreasing ethanol solutions. A 10-s immersion in the thionin solution was followed by differentiation in 70% ethanol and dehydration in 100% ethanol. The tissue was cleared in xylenes, and the slides were coverslipped using DPX (Sigma-Aldrich) as mountant.

Analysis and Photomicrography of Histological Sections. Each section through E.P.'s brain initially was analyzed at low magnification using a Leica MZ8 stereomicroscope. Regions showing changes were examined at higher magnification using a Nikon Eclipse 600 microscope to determine the location, extent, and nature of the changes.

Cytoarchitectonic Analysis. As a preliminary step toward defining the extent of neuropathology in the temporal lobe, a cytoarchitectonic analysis of the temporal lobe cortex of E.P. was carried out. Cytoarchitectonic boundaries followed the nomenclature of Brodmann (2) and Von Economo and Koskinas (3). The medial temporal lobe (mainly the amygdaloid complex and hippocampal formation) was divided into the cytoarchitectonic fields described by Insausti and Amaral (4). For the parahippocampal region, the criteria published by Insausti et al. (5) and Blaizot et al. (6) were followed.

Analysis of Brain Regions Other than the Temporal Lobe. After characterizing primary damage to the medial temporal lobe, serial

sections through the remainder of the brain were evaluated systematically to localize other regions of damage. Areas of cell loss were noted and documented photomicrographically. Sections through the brainstem and cerebellum were analyzed carefully to investigate a parenchymal mass protruding into the fourth ventricle that was revealed during histological processing. This pathological feature was not visible in previous MRI scans (7).

Photomicrographic Documentation of Neuropathology. To illustrate the extent of temporal lobe damage in E.P., low-magnification, photomicrographic montages were created at eight rostrocaudal levels through the temporal lobe using StereoInvestigator software (version 10.5; MBF Biosciences) at 10× magnification. Images of sections from comparable rostrocaudal levels of the control case (H.T.) were imaged using a Creo-Kodak Supreme II – High resolution Flat Bed Scanner (at 5,600 dots per inch; resolution = 4.7 μ per pixel). Illustrations were composed using Adobe Photoshop (v 12.1) on a Macintosh iMac computer. Contrast and levels were adjusted to match all sections, and images were sharpened using the 'Unsharp Mask' filter tool.

1. Rosene DL, Roy NJ, Davis BJ (1986) A cryoprotection method that facilitates cutting frozen sections of whole monkey brains for histological and histochemical processing without freezing artifact. *J Histochem Cytochem* 34(10):1301–1315.
2. Brodmann K (1909) *Vergleinchende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues* (Barth, Leipzig).
3. Von Economo C, Koskinas GN (2008) *Atlas of Cytoarchitectonics of the Adult Human Cerebral Cortex*, ed Triarhou LC (S. Karger, Basel).
4. Insausti R, Amaral DG (2011) Hippocampal formation. *The Human Nervous System*, eds Mai JK, Paxinos G (San Diego, Academic) 3rd Ed, pp 896–942.
5. Insausti R, Insausti AM, Sobreviela MT, Salinas A, Martínez-Peñuela JM (1998) Human medial temporal lobe in aging: Anatomical basis of memory preservation. *Microsc Res Tech* 43(1):8–15.
6. Blaizot X, et al. (2010) The human parahippocampal region: I. Temporal pole cytoarchitectonic and MRI correlation. *Cereb Cortex* 20(9):2198–2212.
7. Stefanacci L, Buffalo EA, Schmolck H, Squire LR (2000) Profound amnesia after damage to the medial temporal lobe: A neuroanatomical and neuropsychological profile of patient E. P. *J Neurosci* 20(18):7024–7036.

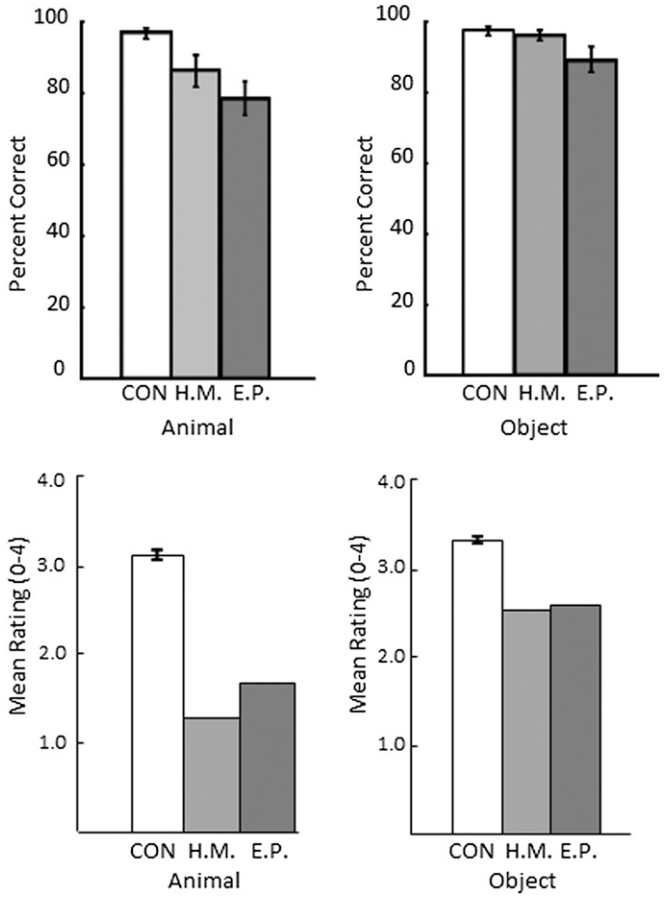

Fig. S1. (*Upper*) Mean performance of patient E.P., patient H.M., and eight control subjects (CON) on six different tests of semantic knowledge about 24 animals (*Left*) and 24 objects (*Right*). For controls, brackets show SEM. For patients, brackets show SEM across the six tests. (*Lower*) Mean performance of patient E.P., patient H.M., and eight controls on two tests that asked participants first to define an item when given its name and in a second test to define the item when shown a drawing of it. (n = 12 animals and 12 objects). The definitions were scored from 0 to 4. Brackets show SEM. (Adapted from ref. 1.)

1. Schmolck H, Kensinger EA, Corkin S, Squire LR (2002) Semantic knowledge in patient H.M. and other patients with bilateral medial and lateral temporal lobe lesions. *Hippocampus* 12(4):520–533.

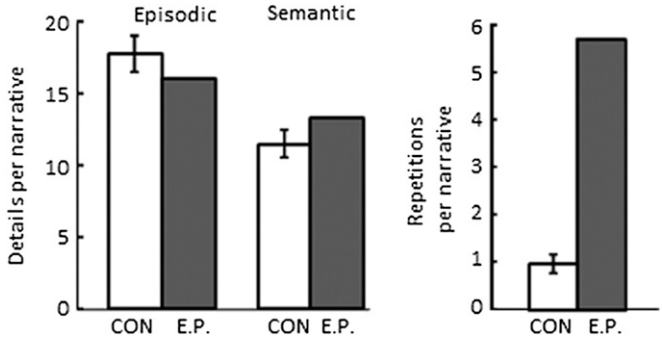

Fig. S2. Number of details contained in remote autobiographical recollections by E.P. and controls ($n = 6$). Participants were given 24 cue words (e.g.,"river," "bottle") and were asked to recollect events related to the cue word from the age of 23 y or earlier (corresponding to the first third of life before E.P.'s amnesia). Each well-formed recollection was scored for narrative content (for E.P., $n = 18$ recollections; for controls, $n = 22.3$ recollections). (*Left*) The number of details that were part of an autobiographical event (episodic memory) and the number of details that were not unique to a specific event (semantic memory). (*Right*) The number of times that participants repeated the same detail in their recollections. Repetitions were not counted in the tally of total details in *A*. Data are from ref. 1.

1. Bayley PJ, Hopkins RO, Squire LR (2003) Successful recollection of remote autobiographical memories by amnesic patients with medial temporal lobe lesions. *Neuron* 37:135–144.

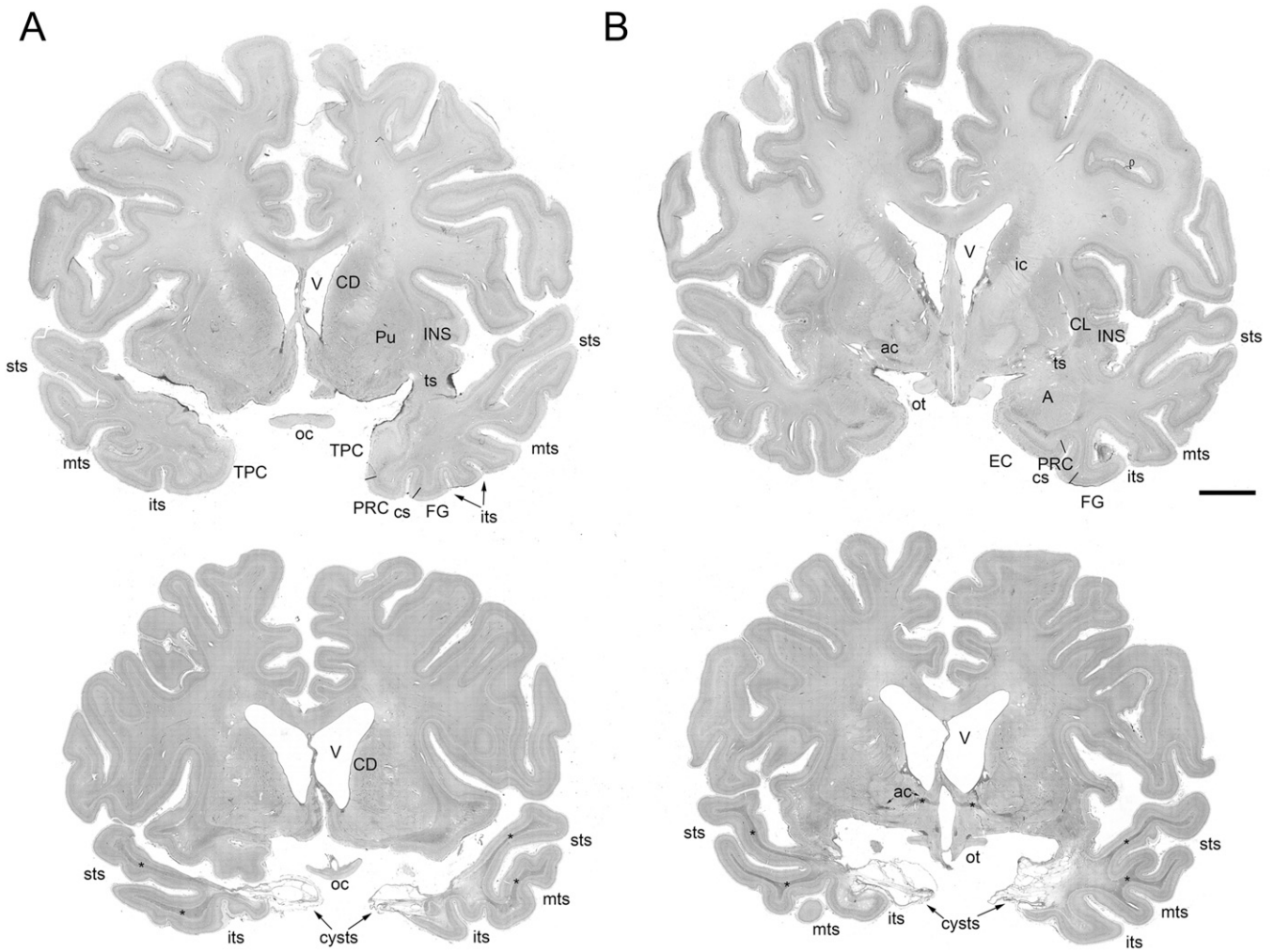

Fig. S3. Figs. S3–S6 present low-power photomicrographs of Nissl-stained sections through the medial temporal lobe in E.P. (*Lower*) and an age-matched control, H.T. (*Upper*). Sections are arranged from rostral to caudal. The area in *A* is at the level of the temporopolar cortex which is heavily damaged bilaterally in patient E.P. The area in *B* is at the level of the rostral amygdaloid complex. It and the surrounding entorhinal and perirhinal cortices are eliminated in E.P. The temporal stem (ts) is also occupied by lateral extensions of the cysts. In general, the temporal neocortex appears to be atrophic, and there is substantial gliosis of the white matter (asterisks). Abbreviations for Figs. S3–S6: A, amygdala; ab, angular bundle; ac, anterior commissure; CD, caudate nucleus; CL, claustrum; cs, collateral sulcus; DG, dentate gyrus; EC, entorhinal cortex; f, fornix; FG, fusiform gyrus; H, hippocampus; ic, internal capsule; INS, insular cortex; its, inferior temporal sulcus; LGN, lateral geniculate nucleus; MMN, medial mammillary nucleus; mts, middle temporal sulcus; oc, optic chiasm; ot, optic tract; PHC, parahippocampal cortex; PRC, perirhinal cortex; Pu, putamen; Pul, pulvinar; sts, superior temporal sulcus; TPC, temporopolar cortex; ts, temporal stem; V, ventricle. (Scale bar: 1 cm.)

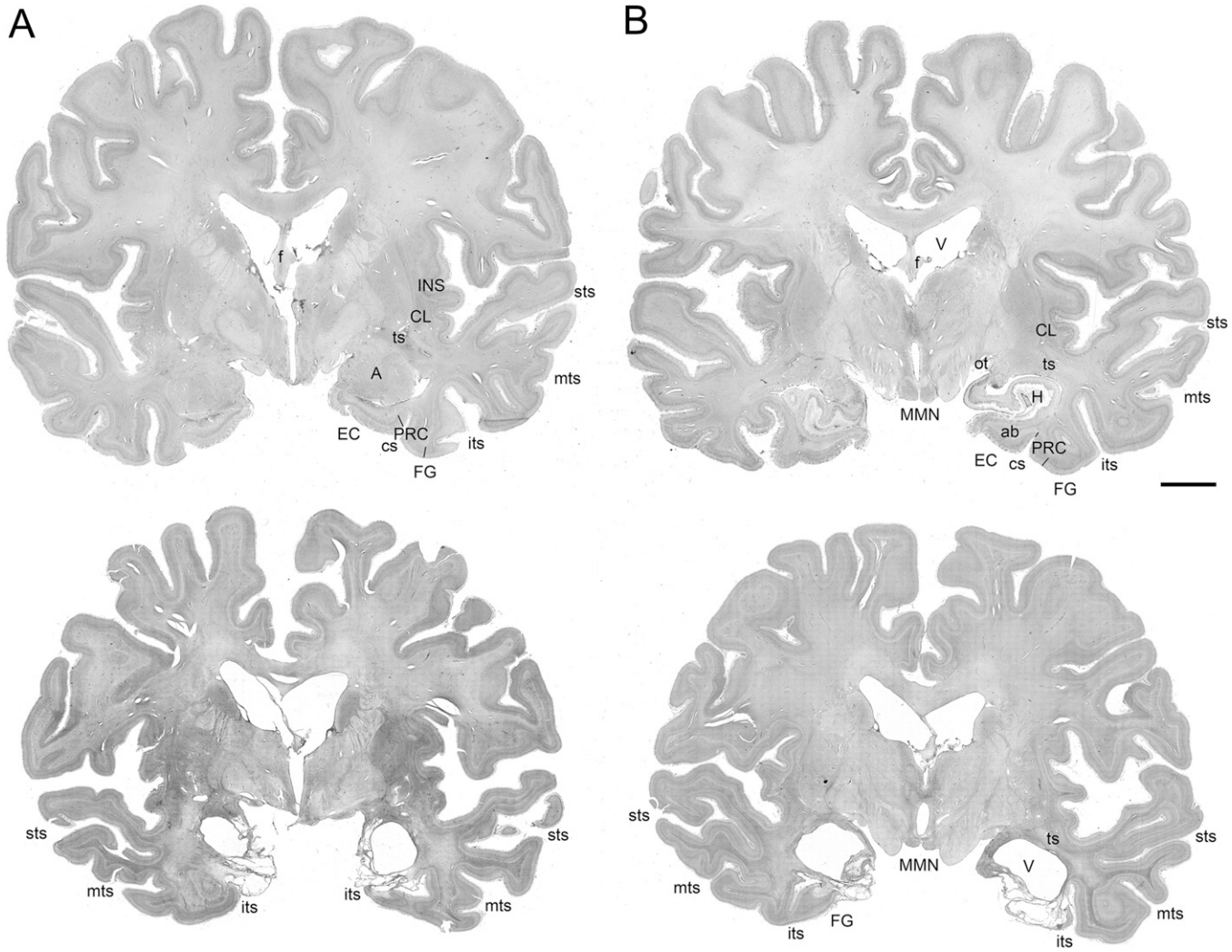

Fig. S4. The area in *A* is through the caudal half of the amygdaloid complex, and it, the entorhinal cortex, and the perirhinal cortex are taken over by cystic cavities. The area in *B* is at the rostral level of the hippocampal formation, which is occupied by cystic cavities in E.P. The ventricle has expanded, and the entorhinal cortex and perirhinal cortex are eliminated bilaterally. See the Fig. S3 legend for abbreviations. (Scale bar: 1 cm.)

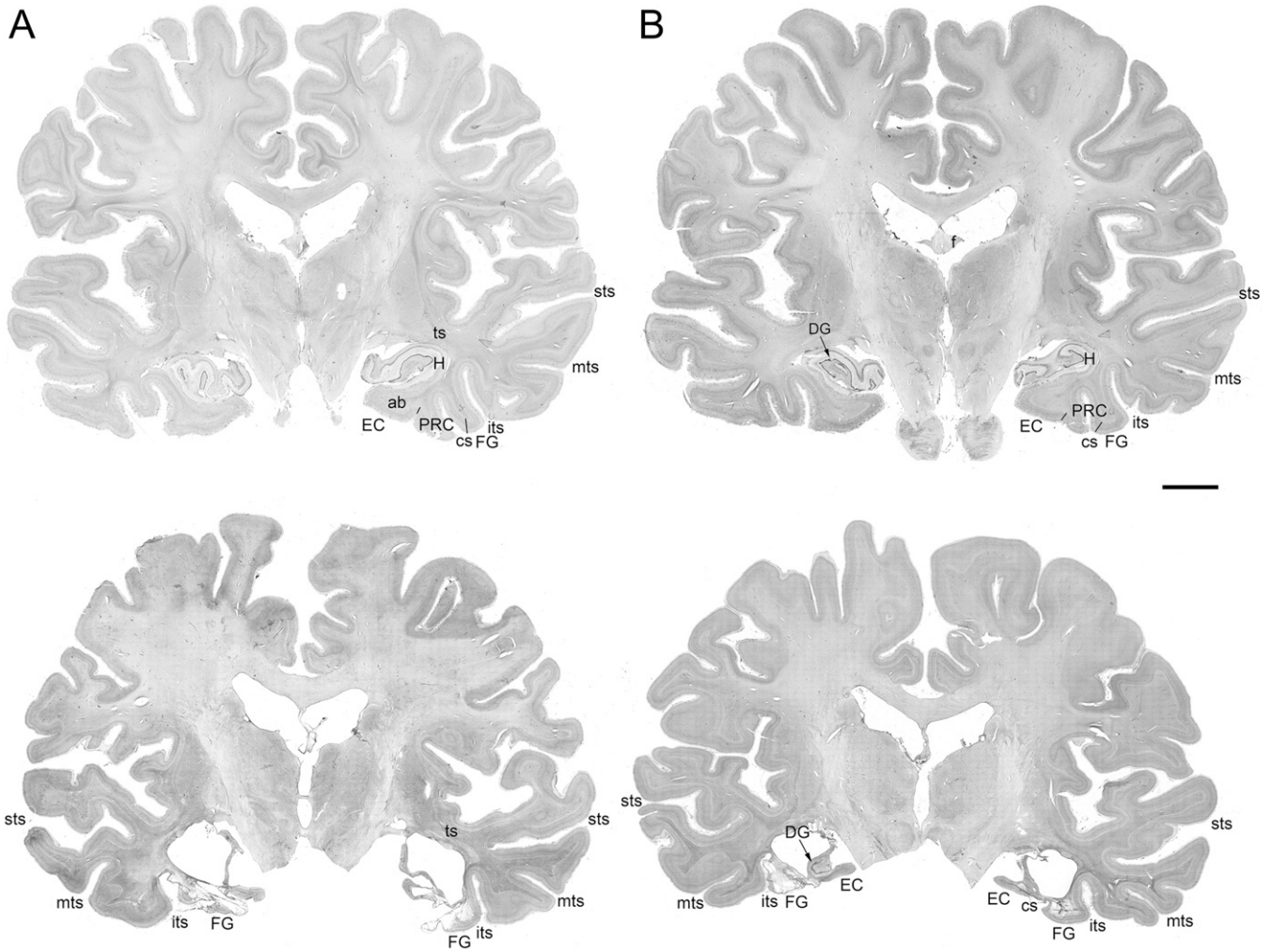

Fig. S5. The areas in *A* and *B* are through successively more caudal levels of the hippocampal formation. Note that almost all the entorhinal cortex is eliminated bilaterally and that the perirhinal cortex is never apparent. See the Fig. S3 legend for abbreviations. (Scale bar: 1 cm.)

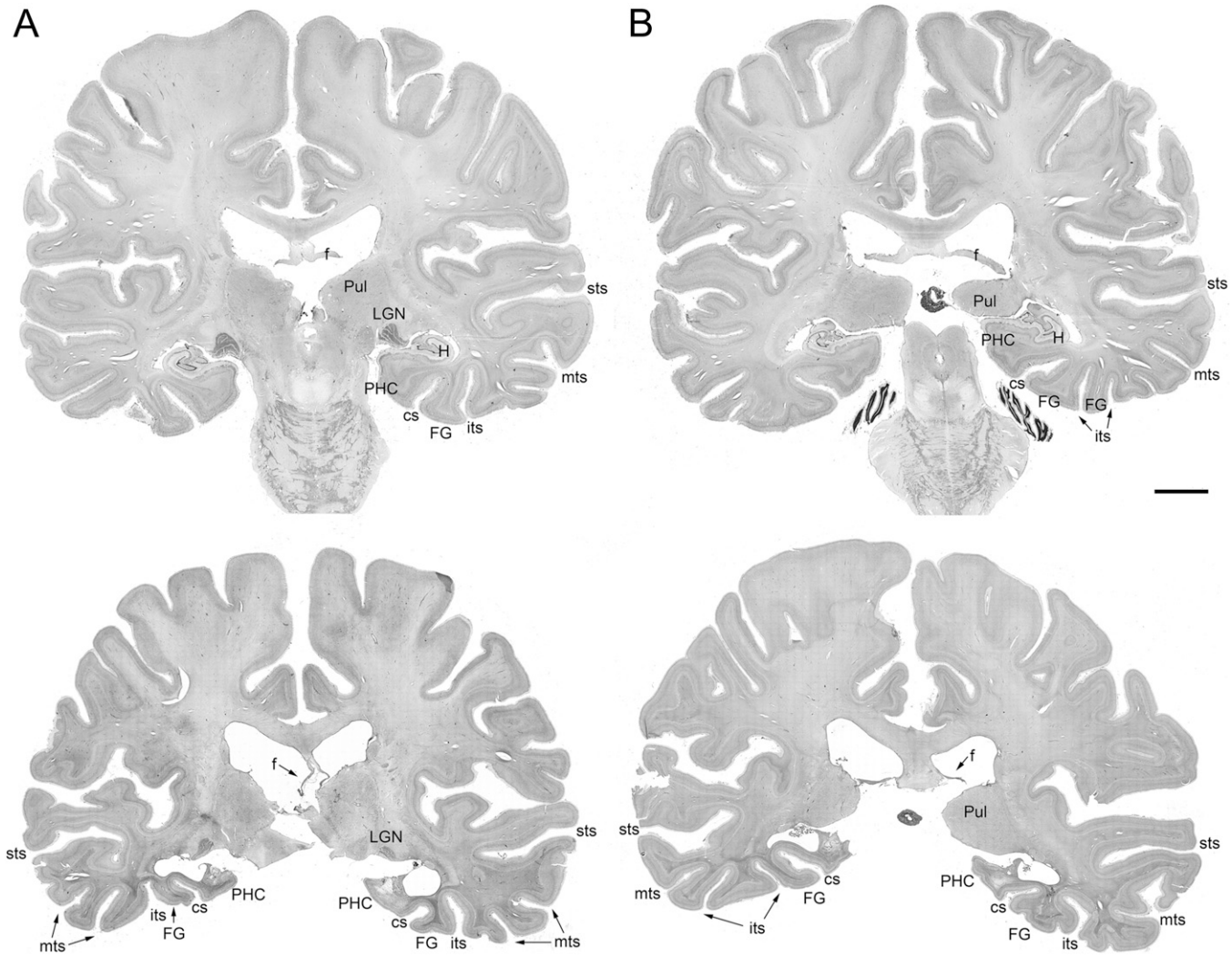

Fig. S6. The areas in *A* and *B* are through the caudal hippocampal formation. The dentate gyrus and hippocampus remain markedly shrunken and gliotic at these levels in E.P. The parahippocampal and fusiform gyrus are present but shrunken. See the Fig. S3 legend for abbreviations. (Scale bar: 1 cm.)

Table S1. Performance on tests of declarative memory

Test (maximum score)	E.P.	Controls (n = 8)
Two-choice recognition memory		
Words (50)	24	48.4
Faces (50)	28	41.9
Complex drawing		
Copy (36)	27	30.3
Recall (36)	0	20.6
Paired-associate learning		
Trial 1 (10)	0	6.0
Trial 2 (10)	0	7.6
Trial 3 (10)	0	8.9

The mean scores for controls for these tests are from Squire and Shimamura (1). The two-choice recognition memory scores are based on a 24-h recognition test of 50 words or 50 faces (maximum score, 50; chance, 25) (modified from ref. 2). The complex drawing score is based on the copy and delayed (10–15 min) reproduction of the Rey–Osterrieth figure (maximum score, 36) (3). The paired-associate scores are the number of word pairs recalled on three successful trials (maximum score, 10 per trial).

1. Squire LR, Shimamura AP (1986) Characterizing amnesic patients for neurobehavioral study. *Behav Neurosci* 100(6):866–877.
2. Warrington EK (1984) *Recognition Memory Test* (NFER-Nelson, Windsor, England).
3. Osterrieth PA (1944) Le test de copie d'une figure complexe. *Arch de Psych* 30:286–356.