Supplementary Table 1

CONTROL

Case No.	Age	Gender	Experimental Use	Clinical History
1	14GW	F	IHC, mapping	spontaneous abortion
2	17GW	M	IHC	amniotic infection
3	17GW	F	IHC	spontaneous abortion
4	22GW	M	IHC	spontaneous abortion
5	22GW	M	IHC, mapping	urethral stenosis
6	22GW	F	IHC,EM	spontaneous abortion
7	27GW	F	IHC	bacterial pneumonia
8	38GW (term)	M	IHC, mapping	Bronchopulmonary dysplasia
9	term	M	IHC	Respiratory failure
10	term	M	IHC	chondrodysplasia
11	term	M	IHC	congenital malformations with diaphragmatic hernia
12	2 days	F	EM	sepsis
13	10 days	F	IHC	atypical motor neuron disease
14	3 weeks	M	IHC, mapping	cardiac mlformations
15	1 month	F	IHC, mapping	cardiovascular malformation
16	6 month	M	IHC, mapping	Tetralogy of Fallot, pneumonia
17	7 month	M	IHC	Wiskott-Aldrich Syndrome
18	1 year	F	IHC, mapping	aspiration pneumonia
19	7 years	M	IHC, EM, mapping	septic shock
20	13 years	M	IHC, EM, mapping	focal segmental glomerulosclerosis
21	18 years	M	EM	unknown
22	19 years	M	IHC	systemic fungal infection
23	25 years	M	EM	unknown
24	35 years	M	IHC, EM, mapping	heart failure
25	36 years	M	IHC	interstitial pulmonary fibrosis
26	39 years	M	IHC	pulmonary failure
27	40 years	F	EM	ACM ictus
28	44 years	F	EM	Cerebral hemorrhage from right carotid aneurysm
29	48 years	M	EM	autoimmune nephritis post transplant
30	52 years	F	IHC	trauma
31	53 years	F	IHC	unknown
32	54 years	M	EM	Spontaneous intraventricular hemorrhage from vascular malformation
33	55 years	F	EM	Secondary spontaneus cerebral intraventricular hemorrhage
34	55 years	M	IHC, mapping	heart failure
35	55 years	M	IHC	liver cancer
36	63 years	M	EM	pancreatic carcinoma
37	77 years	M	IHC	VHL with hemangiomas

Supplementary Table 1

EPILEPSY

Case No.	Age	Gender	Experimental Use	Clinical History
1	3 months	F	IHC	Epilepsy intraoperative resection
2	10 months	M	IHC, mapping	Epilepsy intraoperative resection
3	15 months	M	IHC	Epilepsy intraoperative resection
4	2 years	F	EM	Epilepsy intraoperative resection
5	7 years	M	IHC, mapping	Epilepsy intraoperative resection
6	7 years	F	IHC, EM	Epilepsy intraoperative resection
7	9 years	M	IHC	Epilepsy intraoperative resection
8	10 years	M	IHC	Epilepsy intraoperative resection
9	11 years	M	IHC	Epilepsy intraoperative resection
10	13 years	M	IHC, mapping	Epilepsy intraoperative resection
11	30 years	F	IHC and EM	Epilepsy intraoperative resection
12	30 years	F	IHC	Epilepsy intraoperative resection
13	30 years	M	EM	Epilepsy intraoperative resection
14	31 years	F	IHC and EM	Epilepsy intraoperative resection
15	32 years	F	IHC and EM	Epilepsy intraoperative resection
16	36 years	F	IHC and EM	Epilepsy intraoperative resection
17	46 years	F	IHC and EM	Epilepsy intraoperative resection
18	47 years	F	IHC and EM	Epilepsy intraoperative resection
19	48 years	M	IHC and EM	Epilepsy intraoperative resection
20	49 years	F	IHC and EM	Epilepsy intraoperative resection
21	59 years	M	IHC and EM	Epilepsy intraoperative resection
22	64 years	M	IHC and EM	Epilepsy intraoperative resection

Supplementary Table 2

Animal No.	**Species**	**Age**	**Sex**	**Experimental Use**
1	*M. mulatta*	E150	NA	IHC
2	*M. mulatta*	E150	NA	IHC
3	*M. mulatta*	0 days	M	IHC, EM
4	*M. mulatta*	6 months	M	IHC, EM
5	*M. mulatta*	1.5 years	M	IHC, EM, BrdU
6	*M. mulatta*	1.5 years	M	IHC, EM, BrdU
7	*M. mulatta*	1.5 years	M	IHC, EM, BrdU
8	*M. mulatta*	5 years	M	IHC, EM
9	*M. mulatta*	7 years	M	IHC, EM, BrdU
10	*M. mulatta*	7.5 years	F	IHC, BrdU
11	*M. mulatta*	22 years	F	IHC
12	*M. mulatta*	23.6 years	F	IHC, EM

E=embryonic day

Supplementary Table 3

Primary Ab	Species	Dilution	Manufacturer	Cat. No.	Antigen Retrieval	Lot No.	Product Notes
ALDH1L1	Mouse	1:500	NeuroMab	N103/39	None	N103/31	Species reactivity: Human
Ascl1	Mouse	1:500	BD Pharmingen	556604	1 min	4169563	Reactivity: Rat and Mouse; control tests performed on human tissue
Ascl1	Rabbit	1:2000	Cosmo Bio	SK-T01-003	None	TAK3-002	Species Reactivity: Human
BLBP	Rabbit	1:200	EMD Millipore	ABN14	10 min	2299161	Species Reactivity: Predicted human reactivity based on sequence
BLBP	Mouse	1:200	Abcam	ab131137	10 min	Clone AT1D1	Species Reactivity: Human
BrdU	Rat	1:100	Accurate Chemical	OBT0030	None	H9970	Species reactivity: primate
Doublecortin	Rabbit	1:200	Cell Signaling	4604S	None	42798	Species Reactivity: Human
Doublecortin	Rabbit	1:200	Abcam	ab18723	None	GR324492-1	Species Reactivity: Human
Doublecortin	Guinea pig	1:200	EMD Millipore	AB2253	None	2787730	Species Reactivity: Predicted human reactivity based on sequence
Doublecortin	Goat	1:200	Santa Cruz	SC-8066	None	G1408	Recommended for detection of human doublecortin
GFAP	Chicken	1:750	Abcam	ab4674	None	GR267558-1	Species Reactivity: Human
Hopx	Rabbit	1:200	Sigma-Aldrich	HPA030180	10 min	CC30216	Species Reactivity: Human
Iba1	Goat	1:250	Novus	nb100-1028	None	S7C5P2	Reactivity: Human
Iba1	Rabbit	1:100	Wako	019-1974	None	LKJ2979	Reactive with human Iba1
Ki67	Mouse	1:200	BD Pharmigen	556003	10 min	6110925	QC Testing: Human
Ki67	Rabbit	1:500	Novocastra	NCL-Ki67p	10 min	6029714	Specificity: Human Ki66
Ki67	Rabbit	1:1000	Vector Labs	VP-K451	None	6013873	Specificity: Human Ki67
MCM2	Goat	1:200	Santa Cruz	SC-9839	10 min	D1310	Recommended for detection of human MCM2
Nestin	Mouse	1:250	Covance	MMS-570p	None	14683401	Reactivity: Human
NeuN	Chicken	1:500	EMD Millipore	ABN91	None	2620673	Species reactivity: Mouse, rat; control tests performed on human tissue
NeuN	Rabbit	1:1000	Bioscience	R-3770-100	None	201605-SH	Species Reactivity: raised against human Fox3
NeuroD	Goat	1:50	Santa Cruz	SC-1084	10 min	B0108	Species Reactivity: Human NeuroD
Olig2	Rabbit	1:750	EMD Millipore	AB9610	None	2519344	Species Reactivity: Human
Pax6	Rabbit	1:500	Covance	PRB-279p	10 min	D14BF00330	Species Reactivity: Extensive; control tests performed on human tissue
Prox1	Rabbit	1:500	Chemicon	AB5475	8 min	LV1354325	Species Reactivity: Human
PSA-NCAM	Mouse	1:500	Millipore	MAB5324	None	2201402	Species Reactivity: Human
Sox1	Goat	1:20	R&D	AF3369	8 min	XUV0314121	Species Reactivity: Human
Sox1	Rabbit	1:500	Abcam	ab87775	8 min	GR226877-1	Species Reactivity: Human
Sox2	Goat	1:200	Santa Cruz	sc-17320	10 min	H2914	Species Reactivity: Human Sox2
Tbr2	Rabbit	1:1000	Abcam Inc.	ab23345	None	GR241522-1	Species Reactivity: Human
Tuj1	Mouse	1:200	Covance	MMS-435P	10 min	TU1	Species Reactivity: Human
Vimentin	Mouse	1:1000	Sigma-Aldrich	V5255	None	045K4826	Tested in human appendix/ tonsil

Supplementary Table 4

#	Species	Age	# BrdU Injections	Survival time	# BrdU+ cells	# BrdU+ DCX+ cells	# BrdU+ NeuN+ cells
1	*M. mulatta*	1.5 years	1	2 hours	2.87 ±2.35	0	0
2	*M. mulatta*	1.5 years	10	10 weeks	8.97 ±4.33	2.67 ±2.30	0
3	*M. mulatta*	1.5 years	10	15 weeks	15.22 ±8.52	7.2 ±4.6	0.81 ±0.81
4	*M. mulatta*	7.5 years	10	10 weeks	0.44 ±0.05	0	0
5	*M. mulatta*	7 years	10	15 weeks	1.17 ±1.46	0.07 ±0.26	0

Supplementary Discussion:

Neurogenesis has been shown to continue in adult mammalian brain within two regions V-SVZ and SGZ[36–38]. Young neurons born in the SGZ migrate a short distance and become incorporated in the DG[39]. In contrast, those born in the V-SVZ migrate a long distance to integrate in the olfactory bulb (OB). The extent to which these processes continue in the adult human brain is of considerable interest, given the links to disease, environmental influences, neural plasticity and potentially repair[40,41]. However, the extent to which adult primates[42], and in particular humans, produce and recruit new neurons in adulthood has been controversial. V-SVZ neurogenesis has been shown to continue postnatally in humans, but greatly declines by 1 year of age and is extremely rare in adults[19,20,43,44]. Evidence for the postnatal recruitment of young neurons has also been obtained in the frontal cortex[19,45], but this process also declines rapidly during the first few months of life and the time of birth of these neurons remains unknown. In contrast, adult neurogenesis is thought to continue in the human hippocampus. An essential feature of adult neurogenesis in the DG is the formation of a secondary germinal zone containing neural stem cells (NSCs) within the SGZ[23,24]. In the macaque we found that the architecture of the DG shared some features with the rodent, including a proliferative SGZ at juvenile ages and a layer of young neurons. In the human, however, a proliferative niche of stem cells was not observed within the postnatal SGZ. We do not know if the SGZ forms during the intervening times for which we did not have tissue samples. If this were the case, it would mean that the human SGZ is not a long-lived germinal layer. At 7 and 13 years when it would be expected that a germinal zone is set up for life, we did not observe a coalesced, proliferative SGZ. It is also possible that hippocampal neural stem cells in humans remain dispersed in the hilus. However, if these cells continued to generate neurons in adulthood, we would expect to

see migrating young neurons within the hilus on their way to the GCL, which was not the case.

We do not believe that our inability to detect a proliferative SGZ in humans is due to problems with histological preservation. At young ages in the macaque, there were many cells in the DG with an immature phenotype (little cytoplasm and dark nuclei with several nucleoli; see **Extended Data Fig. 9b**), which were detectable with simple nuclear staining; these cells were not detected in the human (see **Extended Data Fig. 2a**). As a comparison to our autopsy samples, we also examined intraoperative surgical resections from individuals with seizures where fixation occurred within minutes of dissection. Seizures can acutely increase proliferation and young neurons in the rodent SGZ[46,47], but we found no evidence of a discrete layer of dividing cells in the human GCL, the hilus or at its interface in our epileptic cases. Although we could find cells expressing markers of newly formed neurons in the infant and childhood epileptic DG, we did not find these cells in the adult seizure cases. This is consistent with previous reports of decreases in dividing cells, PSA-NCAM+ cells and mature GCL neurons in the epileptic human hippocampus[32,48]. Chronic seizures could deplete the DG of proliferating precursors[49,50], which could interfere with our ability to detect neurogenesis in surgical samples. However, changes in neurogenesis from chronic epilepsy have been shown to disappear by 6 months[51], and the reduction in neurogenesis has been linked to impaired neuronal differentiation, not reduced numbers of dividing cells[52].

One of the earliest studies of adult human DG neurogenesis used a 5 patient cohort that received BrdU and found labeled cells in the GCL[13]. This study quantified BrdU+ cells in different regions of the DG, but it is unclear how many of these labeled cells are neurons. They presented isolated examples of BrdU staining overlapping with NeuN, Calbindin, and neuron

specific enolase (NSE). We do not know if these cells represent rare cases of new neurons that we were unable to reveal in our study, or if they result from BrdU incorporation independent of cell division, or are signals due to the postmortem interval, fixation method, or harsh treatments required for BrdU detection. Intriguingly, these patients received a single dose 1/10th (4-5 mg/Kg) normally used in rodent and monkey studies. We noticed that in our negative controls for BrdU staining in the macaque, or in our non-Brdu treated human tissue, fluorescent round profiles in the SGZ that could be interpreted as labeled cells. Some of this round fluorescence overlapped with DAPI or NeuN (**Extended Data Fig. 7f**). Our BrdU staining in macaques suggests that this method is less sensitive than DCX staining to find the few neurons that continue to be produced after 1.5 years (**Supplementary Table 4**). This is likely due to the long maturation of primate granule neurons providing an extended window of time to detect these adult-born cells by DCX expression. Unfortunately, the tissue treated with BrdU did not have correlative staining with markers of young neurons.

Other studies that immunostained human hippocampus from 11GW to 100 years found evidence for DCX+ cells in all ages studied[14], with a sharp decline in these cells with age; however, only those cells at the youngest ages had elongated morphology consistent with young neurons. A more recent study finds very few Ki67+ cells or DCX+ cells in the adult human hippocampus consistent with our observations[12]. Additional studies[53,54] report DCX+ cells in the adult human DG; however the examples presented display a round nuclei similar to that seen in mature neurons. Furthermore, as seen in our study and by others, some glial cells may express low levels of DCX[26,55]. Therefore, additional markers, such as PSA-NCAM or TUJ1, are important to confirm the neural identity of DCX+ cells.

A study using ^{14}C birthdating on sorted NeuN+ nuclei suggested that hundreds of new neurons are generated per day in the adult human hippocampus, with little decline with age[11]. The results obtained from this method differ from the data presented here and other histology studies that show a sharp decline in markers of newly formed neurons during early postnatal development[12,14]. Birthdating with ^{14}C relies on the isolation of neuronal nuclei using NeuN antibodies, but subpopulations of oligodendrocytes and microglia can also express NeuN[26,55]. ^{14}C could also possibly become incorporated into DNA through methylation or DNA repair independent of cell division, processes that have been shown to occur at higher rates in the hippocampus[56,57]. The proposed addition of new neurons to the adult caudate nucleus using this method[58] is not supported by other work in the human or BrdU labeling in adult macaques[59]. The ^{14}C method is an innovative approach to perform birthdating in postmortem human samples, but it has not been validated in animal studies.

In the postnatal human DG, we do not know when DCX+PSA-NCAM+ cells were born. As we do not have samples between 1 and 7 year of age, we cannot report the level of proliferation during this time. We did detect rare Ki67+ cells at 7 years and 13 years of age, but did not find these dividing cells near young neurons, either in the GCL or in the hilus. The DCX+PSA-NCAM+ cells at these ages often expressed NeuN, and had round nuclei, axons, and highly branched dendrites. It may take months, (possibly years) for new neurons in human to mature and they could maintain DCX and/or PSA-NCAM, well into their maturation. This is consistent with the long neuronal maturation time measured in species with large brains such as the sheep[60,61] and the macaque[62] DG. In the setting of a prolonged maturation, young neurons might therefore be more readily detectable by their DCX expression rather than by their isolation as dividing cells.

It is interesting that in other mammals with large brains, such as dolphins and whales, no young neurons were found in their hippocampus. It is possible that brain size[63] or longevity might constrain neurogenesis. Recent work also suggests that adult neural stem cells self-renew for a limited number of times[64] which could limit the process of neurogenesis to a short period of postnatal life. It has also been previously speculated that long term retention of memories might be incompatible with constant neuronal replacement[65]. Our work highlights the need for caution when considering hypotheses that rely on continued hippocampus neurogenesis as a mechanism, such as in the encoding of new memories. Given the broad interest in adult neurogenesis, we hope our work stimulates further work on the unique development, organization, and function of the human dentate gyrus.

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