

Electrophysiology and Morphology of Human Cortical Supragranular Pyramidal Cells in a Wide Age Range

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In this revised work, Barzó et al. assessed the electrophysiological and anatomical properties of a large number of layer 2/3 pyramidal neurons in brain slices of human neocortex across a wide range of ages, from infancy to elderly individuals, using whole-cell patch clamp recordings and anatomical reconstructions. This large data set represents an **important** contribution to our understanding of how these properties change across the human lifespan, supported by **convincing** data and analyses. The authors have addressed the concerns raised in previous reviews. Overall, this study strengthens our understanding of how the neural properties of human cortical neurons change with age and will contribute to building more realistic models of human cortical function.

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Abstract

The basic excitatory neurons of the cerebral cortex, the pyramidal cells, are the most important signal integrators for the local circuit. They have quite characteristic morphological and electrophysiological properties that are known to be largely constant with age in the young and adult cortex. However, the brain undergoes several dynamic changes throughout life, such as in the phases of early development and cognitive decline in the aging brain. We set out to search for intrinsic cellular changes in supragranular pyramidal cells across a broad age range: from birth to 85 years of age and we found differences in several biophysical properties between defined age groups. During the first year of life, subthreshold and suprathreshold electrophysiological properties changed in a way that shows that pyramidal cells become less excitable with maturation, but also become temporarily more precise. According to our findings, the morphological features of the three-dimensional reconstructions from different life stages showed consistent morphological properties and systematic dendritic spine analysis of an infantile and an old pyramidal cell showed clear significant differences in the distribution of spine shapes. Overall, the changes that occur during development and aging may have lasting effects on the properties of pyramidal cells in the cerebral cortex. Understanding these changes is important to unravel the complex

mechanisms underlying brain development, cognition and age-related neurodegenerative diseases.

Introduction

After birth, the brain undergoes developmental changes for a prolonged time that involve a series of complex and accurately orchestrated processes (Rakic 2009 [↗](#)). The production and migration of neurons is largely complete at the beginning of postnatal development, and then the intrauterine developmental processes continue: gray and white matter thickening, myelination, synaptogenesis, pruning, and establishment of the basic anatomical architecture for initial neural pathway function. Subsequently, local connections within cortical circuits are fine-tuned, and increasingly complex, longer-term connections are established between circuits (Stiles 2010). After that, the changes do not end, but continue throughout human life. They are mostly driven by environmental influences and experiences and lead to changes in metabolic activities (Kuzawa et al. 2014 [↗](#)), changes in functional connectivity patterns (Kelly et al. 2009 [↗](#)) and, with the maturation of white matter (Beck et al 2021 [↗](#), Yeatman et al 2014 [↗](#)) changes in the speed of long-distance transmission (van Blooij et al. 2023 [↗](#)). The final phase is aging, where it slowly declines with advancing age, leading to a decline in cognitive signal processing functions and often resulting in neurodegenerative diseases (Peters 2006 [↗](#)). The cortical supragranular glutamatergic cell (or pyramidal cell) provides the excitatory synaptic inputs for local inhibitory circuitry and other pyramidal cells by which they create distinct subnetworks (Yoshimura 2005). The development and formation of dendrites (Petanjek 2008, Koenderink 1995) and synapses (Huttenlocher and Dabholkar 1997 [↗](#)) of pyramidal cells in the human cerebral cortex has been documented to some extent by postmortem studies, besides much less is known about their biophysical maturation and electrical properties in the early stages of development and the subsequent change or maintenance in later ages. Numerous studies demonstrated in non-primate animal models that the electrical characteristics of neurons change prominently in the early postnatal stage (Molnár et al. 2020 [↗](#)). Changes in the intrinsic membrane properties (Picken Bahrey and Moody 2003 [↗](#)), the input resistance or the kinetics of the elicited action potentials were reported (Kroon 2019, Elston and Fujita 2014 [↗](#)) in connection with maturation of macaque and rodent pyramidal cells. To date, however, no cross-age studies have been conducted on the electrophysiological parameters of human pyramidal neurons. We have studied in detail the postnatal lifetime profile of the physiological and morphological properties of supragranular (layer 2/3) neurons of human pyramidal cells from neurosurgical resections. To this end, we performed whole-cell patch-clamp recordings and 3D anatomical reconstructions of human cortical pyramidal cells from 109 patients aged 1 month to 85 years for comprehensive data analysis to obtain the morphoelectric lifetime profile of supragranular pyramidal cells.

Results

Age-dependent differences in intrinsic subthreshold membrane properties

To extract biophysical properties of excitatory cells of human brain specimens we performed whole cell patch-clamp recordings of pyramidal cells from neurosurgically removed human neocortical tissue sections. The samples were mainly from the frontal and temporal lobes (**Figure 1D** [↗](#), **Supplementary Figure 1D** [↗](#)), mostly from patients with tumors or hydrocephalus (**Supplementary Figure 1B** [↗](#)). Data were collected from 109 patients aged 1 month to 85 years (**Figure 1B-C** [↗](#)) from 498 human cortical layer 2/3 (L2/3) pyramidal cells. To confirm that the studied pyramidal cells originate from the L2/3, we measured the distance between the cell body and the L1 border (Berg 2021). 36% of the cells recovered their soma, with a distance of $129.69 \pm$

130.77 μm from the L1 border (**Fig 1E**). The data set was divided into seven age groups (Kang 2011, Bethlehem 2022): infant: < 1 year, early childhood: 1-6 years, late childhood: 7-12 years, adolescence: 13-19 years, young adulthood: 20-39 years, middle adulthood: 40-59 years, late adulthood: ≥ 60 years (**Figure 1A**).

We evaluated the voltage deflections induced by negative and positive current injections and extracted subthreshold membrane features such as resting membrane potential, input resistance, time constant (τ), and sag ratio in 457 cortical pyramidal cells from 99 patients. We found that the subthreshold features from samples of infant significantly different from those from other age groups (**Figure 2A-D**; resting membrane potential: $P = 3.53 \times 10^{-8}$, input resistance: $P = 1.29 \times 10^{-16}$, τ : $P = 1.31 \times 10^{-15}$, sag ratio: $P = 5.2 \times 10^{-4}$, Kruskal-Wallis test) (**Supplementary Figure 2**). The resting membrane potential was significantly more positive in the first year of life than in the other age groups. Before adulthood a slight decrease was observed in the resting membrane potential across the groups (**Table 1**; **Figure 2A**). Input resistance, τ and sag ratio were measured on voltage deflections elicited by injecting negative (-100 pA) current steps into the cells. A significant decrease in input resistance (**Table 1**) was observed with the largest reduction after the first year of age ($P = 1.88 \times 10^{-6}$, Kruskal-Wallis test with post-hoc Dunn test) (**Figure 2B**). The membrane time constant also decreased significantly in the older groups compared to the infant group, after infancy we found more conserved mean values of membrane time constant into older age (**Table 1**; **Figure 2C**). The ratio of the maximal deflection and the steady-state membrane potential during a negative current step (sag ratio) is significantly higher in late adulthood than in the early stages of life (**Table 1**; **Figure 2D**). Note that the high variance of the infant group data (e.g. resting membrane potential, input resistance, τ) are due to the dynamic change over the 0-1 year period (**Supplementary Figure 3**).

Suprathreshold properties across age-groups

To initiate action potentials we injected positive current steps increased by 20 pA into the cells and recorded various types of input-output transformations. We extracted 25 features from different action potentials (AP) and firing patterns and assessed active membrane properties from recordings filtered for appropriate electrophysiological quality (see Methods). We found that the infant group differs most from the other age groups in several of the suprathreshold properties (**Figure 3**), but other trends are also apparent (rheobase current: $P = 8.71 \times 10^{-12}$, AP half-width: $P = 9.57 \times 10^{-25}$, AP up-stroke velocity: $P = 1.63 \times 10^{-12}$, AP amplitude: $P = 2.24 \times 10^{-11}$, Kruskal-Wallis test) (**Supplementary Figure 4**). For example, the average rheobase current, the minimum current that can trigger an action potential, is significantly lower in the early ages of life than in data collected from adolescence stage (**Table 2**) (infant vs. adolescence $P = 4.15 \times 10^{-13}$, Kruskal-Wallis test with post-hoc Dunn test). Further on the age scale we found that rheobase current was increased with age reaching a maximum value at the adolescent ages then declining to a significantly lower level (adolescent vs. late adulthood, $P = 5.23 \times 10^{-4}$, Kruskal-Wallis test with post-hoc Dunn test, **Figure 3A**). The action potential (AP) half-width averages among a declining trend through the groups of age (**Table 2**) varied considerably across age groups forming significant differences between childhood and adulthood ages (**Figure 3B**). Action potential up-stroke velocities were significantly slower in the infant APs than in all the other ages (**Table 2**; **Figure 3C**). The amplitude of the elicited APs also showed age-dependent differences between age groups (**Table 2**); in the first year of life, the amplitude of APs were significantly lower than in other age groups. In adulthood, a significant decrease with age was observed (**Figure 3D**). Electrophysiological differences markedly separated the infant group from the older age groups shown on UMAP projection (**Figure 3F**, **Supplementary Figure 5**).

Next, we investigated how somatic current inputs are transformed to action potential output by evaluating the firing patterns of cells evoked by injecting prolonged positive current steps (**Figure 4A**) (**Supplementary Figure 6**). The neurons ($n = 331$) were regular spiking cells with moderate adaptation. The slope of the firing frequency versus the current curve (f-I slope) showed

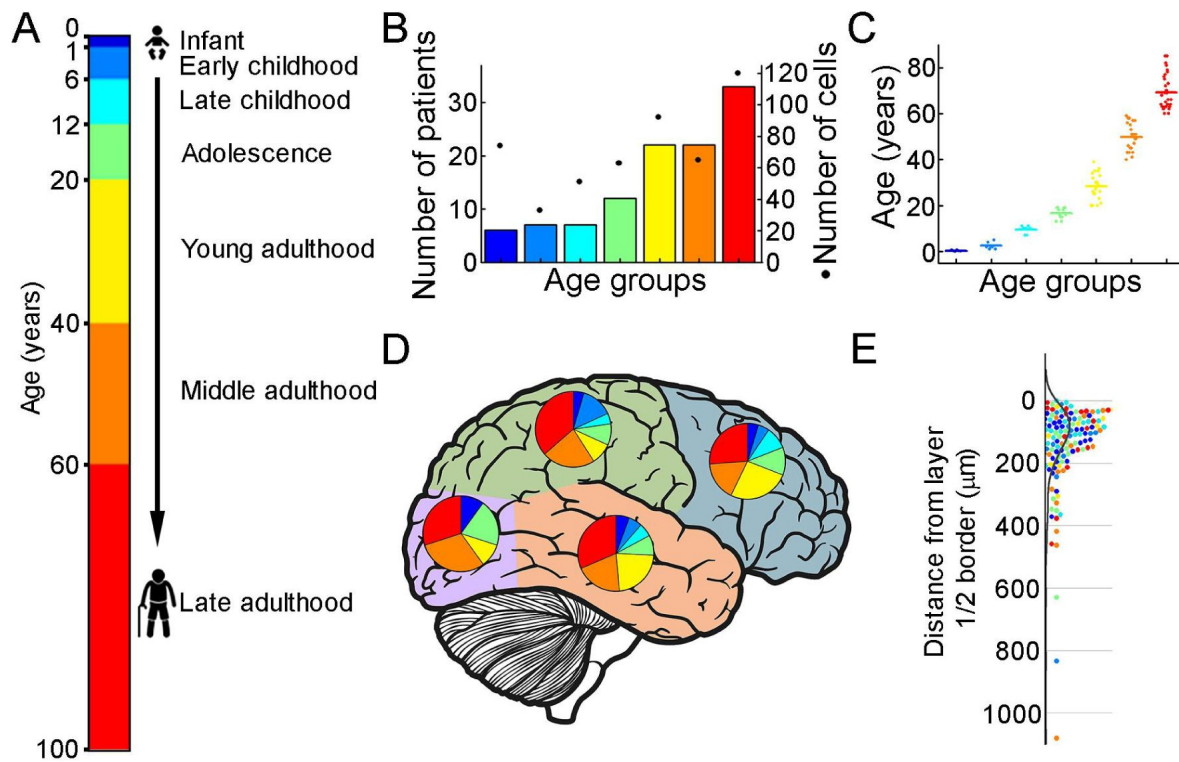


Figure 1.

Illustration of the patient data on the samples utilized

(A) Illustration of the defined age groups.

(B) Number of patients involved in age groups, ($n = 6, 7, 7, 12, 22, 22, 33$ from infant to late adulthood, respectively). Dots show the number of human layer 2/3 pyramidal cells in our dataset regarding the defined age groups ($n = 74, 33, 51, 63, 92, 66, 120$ from infant to late adulthood, respectively).

(C) Distributions of patient ages within age groups.

(D) Brain model indicates the number of surgically removed tissues from the cortical lobes. Colors indicate age groups.

(E) The distribution of recovered cell bodies distance from the L1/2 border.

Figure 2.

Subthreshold membrane properties vary across life stage

(A-D), Boxplots show resting membrane potential (A), input resistance (B), tau (C), and sag ratio (D) distributions in various age groups. (B) Inset shows representative voltage traces from each group. Scale bar: 5 mV; 20 ms. Asterisks indicate significance (Kruskal-Wallis test with post-hoc Dunn test, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

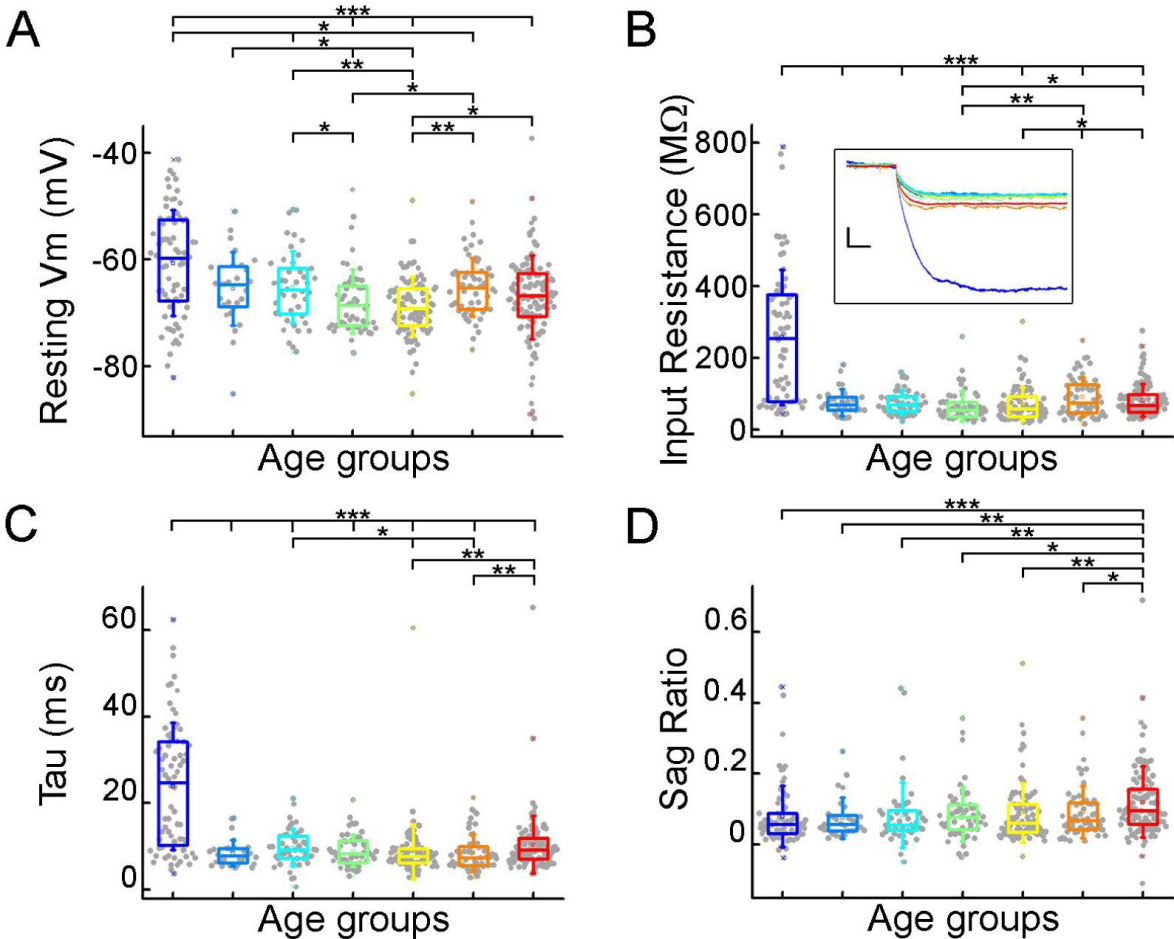


Table 1.

Subthreshold membrane properties

Table showing the mean and standard deviation of the passive parameters across the age groups.

Subthreshold properties	Infant N = 72	Early childhood N = 28	Late childhood N = 45	Adolescence N = 54	Young adulthood N = 89	Middle adulthood N = 56	Late Adulthood N = 113
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Resting Vm (mV)	-60.64 ± 9.86	-65.44 ± 6.82	-65.17 ± 6.68	-67.86 ± 5.94	-68.69 ± 5.54	-65.14 ± 5.5	-67.05 ± 7.86
Input resistance(MΩ)	257.25 ± 188.06	75.27 ± 37.5	74.61 ± 34.29	64.79 ± 41.82	70.45 ± 46.9	90.14 ± 54.37	81.14 ± 46.36
Tau (ms)	23.88 ± 14.7	8.49 ± 3.08	9.73 ± 4.24	8.99 ± 3.71	8.76 ± 6.33	8.53 ± 4.24	10.39 ± 6.63
Sag ratio	0.079 ± 0.87	0.075 ± 0.058	0.082 ± 0.092	0.086 ± 0.076	0.09 ± 0.084	0.092 ± 0.073	0.12 ± 0.1

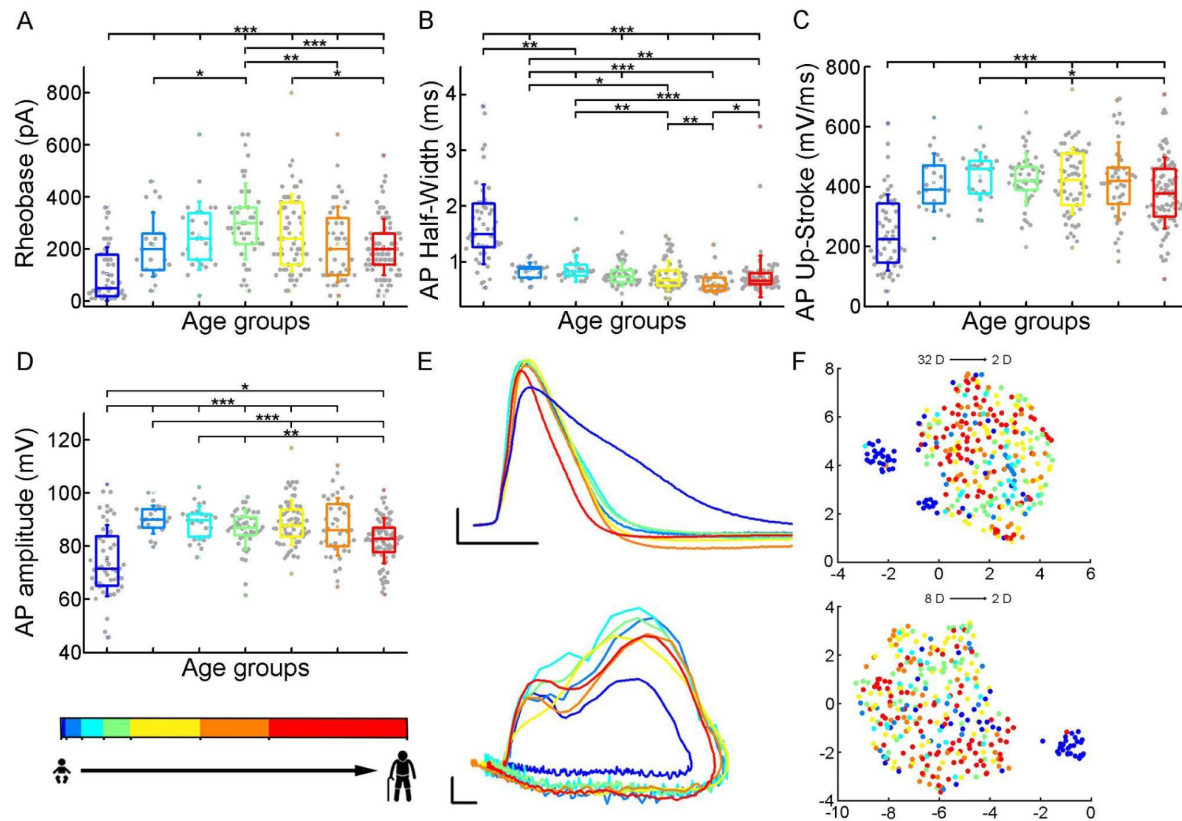


Figure 3.

Age related differences in the action potential kinetics.

(A-D) Boxplots show differences in rheobase (A), action potential half-width (B), action potential up-stroke (C), and action potential amplitude (D) between the age groups. Asterisks indicate statistical significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

(E) Representative action potentials aligned to threshold potential onset (scale: x axis: 1ms, y axis: 20 mV) (top) and phase plots of the representative APs (scale: x axis: 10 mV, y axis: 100 mV/ms) (bottom).

(F) Uniform Manifold Approximation and Projection (UMAP) of 32 (Table 4) (top) and 8 selected electrophysiological properties (resting V_m , input resistance, tau, sag ratio, rheobase, AP half-width, AP up-stroke and AP amplitude) (bottom) with data points for 331 cortical L2/3 pyramidal cells, colored with the corresponding age groups.

Table 2.

Action potential and firing pattern parameters

Table showing the mean and standard deviation of the suprathreshold properties across the age groups.

Suprathreshold properties	Infant N = 51	Early childhood N = 21	Late childhood N = 25	Adolescence N = 45	Young adulthood N = 63	Middle adulthood N = 43	Late Adulthood N = 83
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Rheobase (pA)	104.51 $\pm$ 103.18	218.1 $\pm$ 123.27	252.8 $\pm$ 131.17	306.22 $\pm$ 147.21	262.9 $\pm$ 148.06	219.07 $\pm$ 145.52	207.71 $\pm$ 107.83
AP half-width (ms)	1.68 $\pm$ 0.71	0.84 $\pm$ 0.15	0.88 $\pm$ 0.24	0.78 $\pm$ 0.22	0.76 $\pm$ 0.27	0.62 $\pm$ 0.17	0.74 $\pm$ 0.38
AP up-stroke (mV/ms)	247.43 $\pm$ 127.27	413.68 $\pm$ 97.11	434.75 $\pm$ 78.32	424.69 $\pm$ 87.58	418.49 $\pm$ 110.33	419.15 $\pm$ 129.29	379.22 $\pm$ 118.45
AP amplitude (mV)	74.62 $\pm$ 13.4	90 $\pm$ 5.23	88.82 $\pm$ 6.35	86.31 $\pm$ 7.36	88.81 $\pm$ 8.52	87.28 $\pm$ 10.77	82.02 $\pm$ 8.49
F-I slope (Hz/pA)	0.142 $\pm$ 0.137	0.152 $\pm$ 0.066	0.144 $\pm$ 0.071	0.139 $\pm$ 0.141	0.127 $\pm$ 0.08	0.176 $\pm$ 0.113	0.165 $\pm$ 0.117
First AP latency (ms)	161.26 $\pm$ 76.81	121.36 $\pm$ 76.88	115.29 $\pm$ 81.27	136.69 $\pm$ 109.78	132.42 $\pm$ 84.68	140.61 $\pm$ 143.43	106.39 $\pm$ 52.31
Adaptation	0.103 $\pm$ 0.087	0.061 $\pm$ 0.062	0.068 $\pm$ 0.06	0.105 $\pm$ 0.1	0.119 $\pm$ 0.118	0.114 $\pm$ 0.153	0.124 $\pm$ 0.1

Table 3.

Morphological properties

Table showing the mean and standard deviation of the morphological characteristics across the age groups.

Morphological properties	Infant N = 7	Early childhood N = 8	Late childhood N = 11	Adolescence N = 11	Young adulthood N = 9	Middle adulthood N = 9	Late adulthood N = 8
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Total dendritic length (mm)	6.74 $\pm$ 2.56	8.5 $\pm$ 4.56	6.62 $\pm$ 2.29	9.27 $\pm$ 4.82	6.76 $\pm$ 2.9	8.7 $\pm$ 2.7	8.96 $\pm$ 3.22
Apical dendritic length (mm)	3.77 $\pm$ 1.24	4.57 $\pm$ 2.42	3.78 $\pm$ 1.14	5.24 $\pm$ 2.51	3.88 $\pm$ 1.8	4.52 $\pm$ 1.63	4.64 $\pm$ 1.25
Basal dendritic length (mm)	2.97 $\pm$ 1.63	3.93 $\pm$ 2.74	2.84 $\pm$ 1.33	4.04 $\pm$ 2.44	2.88 $\pm$ 1.27	4.18 $\pm$ 1.35	4.31 $\pm$ 2.13
Total number of nodes	37.42 $\pm$ 23.58	40.88 $\pm$ 20.54	35.36 $\pm$ 12.07	48.36 $\pm$ 24.23	32.11 $\pm$ 12.38	47.33 $\pm$ 15.57	48.38 $\pm$ 16.47
Max. horizontal extension (μ m)	403.33 $\pm$ 118.55	455.24 $\pm$ 114.42	417.2 $\pm$ 98.1	472.2 $\pm$ 104.51	448.3 $\pm$ 75.94	393.7 $\pm$ 90.29	420.93 $\pm$ 77.49
Max. vertical extension (μ m)	498.12 $\pm$ 87.85	521.21 $\pm$ 102.88	463.83 $\pm$ 94.63	529.58 $\pm$ 129.28	447.41 $\pm$ 114.17	488.02 $\pm$ 81.02	490.45 $\pm$ 100.53
Apical terminal length (μ m)	182.42 $\pm$ 48.17	204.83 $\pm$ 49.62	162.47 $\pm$ 21.5	189.59 $\pm$ 42.54	199.68 $\pm$ 36.2	151.23 $\pm$ 23.3	174.62 $\pm$ 36.76
Basal terminal length (μ m)	142.54 $\pm$ 32.43	142.69 $\pm$ 30.31	138.7 $\pm$ 31.22	150.32 $\pm$ 26.09	155.29 $\pm$ 32.15	131.6 $\pm$ 30.44	148.96 $\pm$ 38.42

Resting Vm	The membrane potential of the neuron, measured directly after attaining the whole-cell configuration with no current (if a holding current was used during the recording we compensated the resting membrane potential with the injected current).
Input resistance	To calculate input resistance the mean of all hyperpolarizing current produced voltage steps were used.
Tau	To calculate time constant the mean of all hyperpolarizing current produced voltage steps were used, measured between 0-63%.
Sag ratio	The ratio of the maximal deflection and the steady-state membrane potential during a -100 pA current step.
Rheobase	The minimal current step that elicited the first spike.
AP half-width	The width of the AP at half amplitude.
AP up-stroke	The mean of all the maximum values of dV/dt between the action potential onset and the action potential peak from each elicited APs of the cell.
AP amplitude	Average amplitude of all APs, from threshold to peak.
F-I slope	The slope of the line fitted to the data of the AP firing frequency versus stimulus intensity.
First AP latency	The duration from the start of the stimulus until the first AP under the rheobasic current step.
Adaptation	The average adaptation of the interspike interval between consecutive APs.
Rebound	The difference between the steady-state membrane potential and the maximum deflection after a hyperpolarizing current step.
Rebound-Sag ratio	The ratio of the rebound and sag amplitudes.
Avg. AP number	Average number of elicited APs per sweep.
AP threshold	Mean of all the voltage values at AP threshold.
Ap rise time	Mean time between the threshold and the peak of all APs.
AHP amplitude	Average amplitude of all afterhyperpolarization.
AHP length	Average duration of 0 (AHP minimum) to 90% of all the AHP.
Voltage at max. dV/dt	Average voltage value at the maximum of the AP dv/dt over all APs
Velocity at min. dV/dt	Average velocity value at the minimum of the AP dv/dt from all APs
Voltage at min. dV/dt	Average voltage value at the minimum of the AP dv/dt from all APs
AP peak	Average of AP maximum voltages
ISI mean	Average interspike interval from all sweeps containing at least three APs
AP amplitude accommodation	The difference of the first and last AP amplitude in a sweep.
AP half-width accommodation	The difference of the first and last AP half-width in a sweep.
AP threshold accommodation	The difference of the first and last AP threshold in a sweep.
Average ISI	The mean value of interspike intervals in a sweep.
AP amplitude adaptation	The average adaptation of the AP amplitude between consecutive APs.
AP half-width adaptation	The average adaptation of the AP half-width between consecutive APs.
AP threshold adaptation	The average adaptation of the AP threshold between consecutive APs.
AHP area	Integral of the AHP from the minimum value to 90%, using trapezoidal method.
ADP amplitude	The amplitude of the afterdepolarization, the average difference between the minimum value of the AHP and the threshold of the next AP.

Table 4.

Examined electrophysiological properties

no significant difference across the age groups ($P = 0.055$, Kruskal-Wallis test) (Table 2; Figure 4B). Age related difference was observed in the latency of the first AP during rheobase current injection (First AP latency), in the first year of life the latency of the first spike is significantly higher than all the other groups of age ($P = 7.67 \times 10^{-4}$, Kruskal-Wallis test), which is the most prominent with the oldest age group ($P = 8.41 \times 10^{-6}$, Kruskal-Wallis test with post-hoc Dunn test) (Table 2; Figure 4C). The adaptation of the AP frequency response to the same current injection stimulus also showed differences ($P = 0.032$, Kruskal-Wallis test) between the younger and the groups older than 13 years patients, we found the lowest adaptation values in early childhood (Table 2; Figure 4D).

Previous research has shown that the biophysical properties of the human pyramidal cells show depth-related correlations throughout the L2/3 (Berg 2021, Kalmbach 2018, Chameh 2021). Although there are some deeper cells in our dataset, the majority comes from the upper region of the L2/3. We compared the electrophysiological characteristics according to their depth from the border of L1 and L2 to exclude the possibility that the biophysical differences we found were a result of depth dependence. We did not find any overall differences related to distance of the soma from the L1 border within the age groups with a few exceptions. For example, the values of input resistance ($P = 0.02$, Mann-Whitney test) and AP up-stroke velocity ($P = 0.04$, Mann-Whitney test) differ significantly in the middle adulthood group. We found a significant difference in AP amplitude ($P = 0.02$, Mann-Whitney test) and adaptation ($P = 0.009$, Mann-Whitney test) in the adolescence age group (Supplementary Figure 7-9).

Morphological features of layer 2/3 pyramidal cells in different stages of life

To investigate possible morphological differences between the age groups, we filled the pyramidal cells with biocytin during recordings. Only neurons with no signs of deterioration and with complete apical dendrites and no signs of truncated dendritic branches or tufts were considered for morphological analysis. 63 pyramidal cells (Supplementary Figure 10) were reconstructed in 3D at ages 0-73 years (infant $n = 7$, early childhood $n = 8$, late childhood $n = 11$, adolescence $n = 11$, young adulthood $n = 9$, middle adulthood $n = 9$, late adulthood $n = 8$) (Supplementary Figures 11-12). Figure 5A shows examples of the reconstructed pyramidal cells. We did not detect significant change in total dendritic length ($P = 0.37$, Kruskal-Wallis test) (Table 3; Figure 5B), apical dendritic length ($P = 0.6$, Kruskal-Wallis test) (Table 3; Figure 5C), or basal dendritic length ($P = 0.28$, Kruskal-Wallis test) (Table 3; Figure 5D) at different ages. To investigate dendritic complexity we measured the total number of dendritic branching and found no significant developmental changes ($P = 0.18$, Kruskal-Wallis test) (Table 3; Figure 5E). We also did not observe significant differences in the size of dendritic branching when we measured the maximum horizontal ($P = 0.64$, Kruskal-Wallis test) (Table 3; Figure 5F) and vertical ($P = 0.51$, Kruskal-Wallis test) (Table 3; Figure 5G) extent of the reconstructed cells. We found significant differences in the average length of the uncut terminal segments of apical ($P = 0.033$, Kruskal-Wallis test) (Table 3; Figure 5H) but not of the basal dendrites ($P = 0.85$, Kruskal-Wallis test) (Table 3; Figure 5I) of the cells across the age groups.

To analyze the distribution of dendritic spines we identified and labeled each spine on $n = 6$ fully 3D-reconstructed cells (Figure 6). We compared the spine density of selected pyramidal cells of two age groups: infant (83 days old, $n = 3$ of one patient, parietal lobe) vs. late adulthood (64.3 $\pm$ 2.08 years old, $n = 3$ of 3 patients, frontal, temporal and parietal lobes). The investigated cells are located in L2 (infant: $144.43 \pm 45.26 \mu\text{m}$, late adulthood: $161.22 \pm 66.22 \mu\text{m}$). We found that the total spine density was higher ($P = 7.57 \times 10^{-40}$, Mann-Whitney test) and also the spine density of both apical ($P = 2.02 \times 10^{-31}$, Mann-Whitney test) and basal ($P = 3.8 \times 10^{-12}$, Mann-Whitney test) dendrites was higher in the infant than in the late adult group (Figure 6B). To evaluate the age-dependence of spine morphology, we classified the spines into commonly used phenotypes based on their morphological characteristics (Li 2023), specifically distinguishing between mushroom-

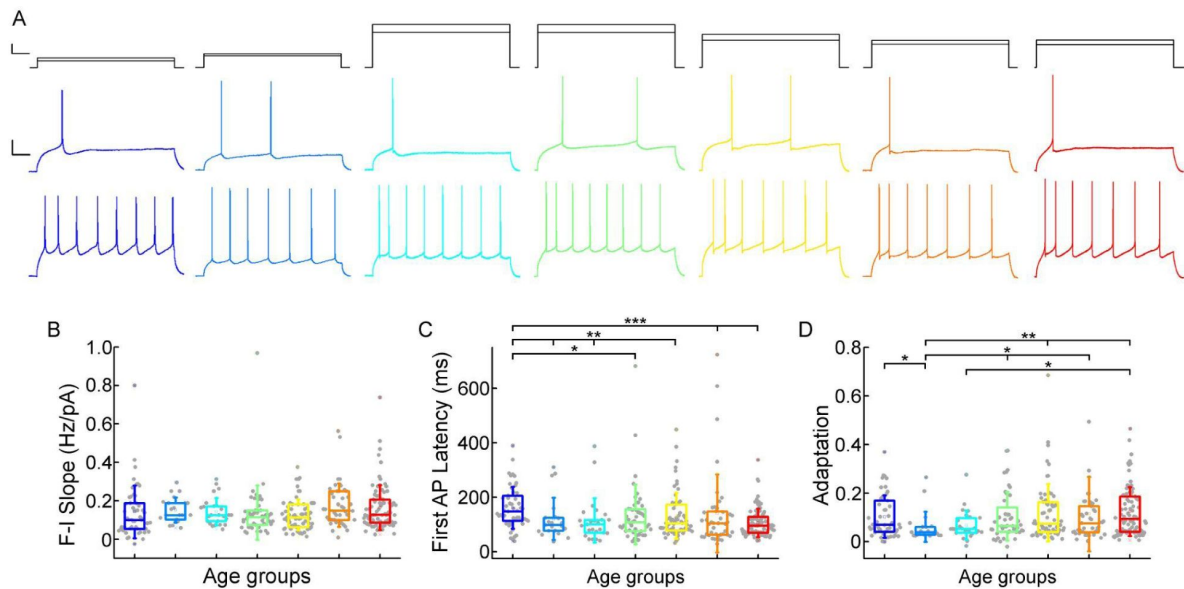


Figure 4.

Age-dependency of the AP firing pattern parameters

(A) Representative membrane potential responses to an 800 ms long rheobase (middle) (left to right: infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood, late adulthood), and increased current steps (bottom) Colored respectively to the age groups. Scale bar top: 1ms, 100 pA, bottom: 1ms, 20mV.
 (B-D) Boxplots show changes across the age groups in f-I slope (B), first AP latency (C), and adaptation of APs (D). Asterisks indicate statistical significance (* P < 0.05, ** P < 0.01, *** P < 0.001).

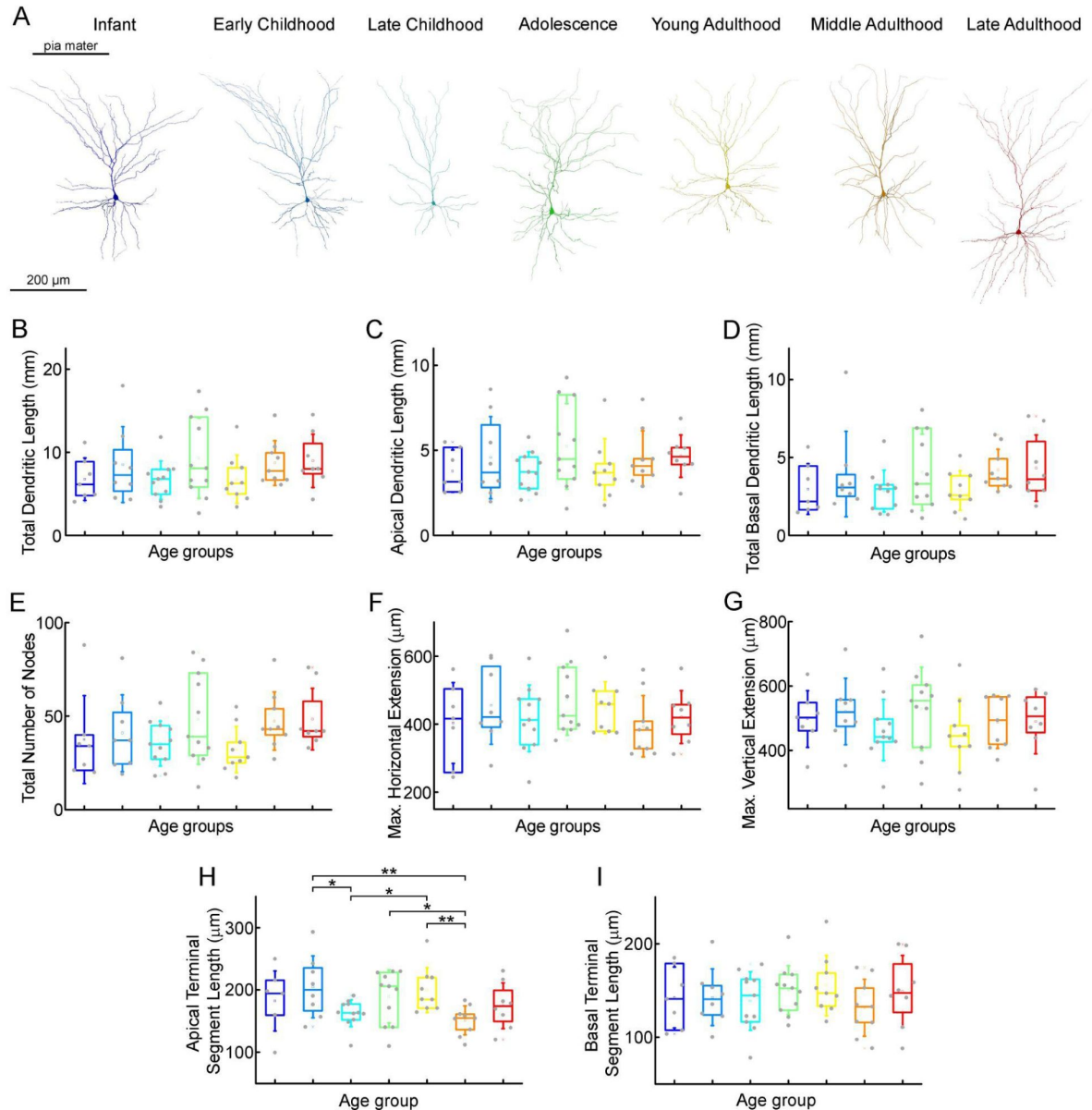


Figure 5.

Morphological features of layer 2/3 pyramidal cells in different stages of life

(A) Representative reconstructions of L2/3 pyramidal cells (from left to right) from infant (n = 7), early childhood (n = 8), late childhood (n = 11), adolescence (n = 11), young adulthood (n = 9), middle adulthood (n = 9), and late adulthood (n = 8) patients.

(B-I) Boxplots show summarized data from all the reconstructed cells (Suppl. Fig. 7) of total dendritic length (B), apical dendritic length (C), total basal dendritic length (D), the total number of nodes on the apical and basal dendrites (E), the maximal horizontal (F), and the maximal vertical (G) extension of dendrites, the average length of the apical (H) and basal (I) terminal dendritic segments. Asterisks indicate statistical significance (* $P < 0.05$, ** $P < 0.01$, Kruskal-Wallis test with post-hoc Dunn test).

shaped, thin, filopodial, branched, and stubby spines (**Figure 6C-G**). Spines with large spine heads were classified as mushroom-shaped, those with small heads as thin, and long protrusions were distinguished as filopodial. Those that did not have peduncles were classified as stubby, spines with two heads emerging from the same spot were called branched spines (**Figure 6C-G**) (Luebke 2015). Only fully visible spines were included in the classification analysis. The composition of spine types varies between the two age groups, mushrooms are present at a higher percentage on apical branches in late adulthood cells ($P = 4.4 \times 10^{-9}$, Mann-Whitney test), and on the basal processes ($P = 9.04 \times 10^{-8}$, Mann-Whitney test) (**Figure 6C,H**). In contrast, thin spines and filopodia are present in significantly higher numbers on the apical (**Figure 6D-E**) (thin spines: $P = 7.34 \times 10^{-14}$, filopodia: $P = 1.11 \times 10^{-39}$, Mann-Whitney test) and basal (thin: $P = 2.46 \times 10^{-8}$; filopodia: $P = 2.14 \times 10^{-12}$, Mann-Whitney test) (**Figure 6I-J**) dendritic branches of the infant. Both apical and basal infant branches had a significantly higher percentage of branched spines (apical: $P = 1.64 \times 10^{-11}$; basal: $P = 8.9 \times 10^{-5}$, Mann-Whitney test) (**Figure 6F,K**), which were present in modest numbers on both. Stubby spines were also more prevalent on the elderly pyramidal cells, either on the apical ($P = 7.19 \times 10^{-5}$, Mann-Whitney test) or basal ($P = 6.97 \times 10^{-9}$, Mann-Whitney test) processes (**Figure 6G,L**). Comparing the spine density of the six individual cells, we found differences across the two age groups similar to those previously mentioned, alongside slight variation within age groups. (**Supplementary Figure 13**, Kruskal-Wallis test with post-hoc Dunn test).

Cortical tissue was dissected during neurosurgical procedures for various pathologies, but not to the same extent in the different groups. Specimen collection in adult/elderly patients was mostly for tumor resection, whereas in children hydrocephalus was the most common reason for brain surgery (**Supplementary Figure 1**). To determine the difference in medical condition could contribute to age-related differences in cellular features, we compared the extracted electrophysiological and morphological features based on the medical condition in the different age groups. It should be noted that all circumstances of surgical steps, tissue dissection, transport (time, media, temperature etc.) and cutting procedure remained the same in the different conditions. When comparing passive electrophysiological properties, we found no significant differences between the different medical conditions in the age groups from infancy to middle adulthood, only time constants were found to be significantly lower in the hydrocephalus patients than in the tumor patients in the young adulthood ($P = 0.048$, Mann-Whitney test) and late adulthood groups ($P = 0.01$, Mann-Whitney test) (**Supplementary Figure 14**). Comparing the action potential kinetics and firing pattern-related parameters between the different pathology groups we found no overall differences with some sporadic exceptions. For example, the parameters of AP half-width in young adulthood ($P = 0.01$, Mann-Whitney test) and the values of rheobase in early childhood ($P = 0.04$, Mann-Whitney test) differed significantly between the different pathologies (**Supplementary Figure 15**). Regarding the firing pattern-related properties we found significant changes in the infant F-I slope ($P = 0.02$, Mann-Whitney test), first AP latency ($P = 0.006$, two sample t-test) and adaptation ($P = 0.002$, Mann-Whitney test) parameters. Also in the late adulthood group the F-I slope ($P = 0.04$, Mann-Whitney test), the first AP latency ($P = 0.02$, Mann-Whitney test) and the adaptation ($P = 0.047$, Mann-Whitney test) were significantly different between the hydrocephalus and tumor patients (**Supplementary Figure 16**). Further comparison of morphological features revealed no statistical difference between tumor and hydrocephalus groups (**Supplementary Figure 17**).

To ascertain whether age-related variations in cell properties may be influenced by the gender of the patient, we compared the examined characteristics of the cells by their sex (**Supplementary Figure. 18-21**). Comparing the passive membrane properties of the cells in different age groups we found higher resting membrane potential values in adolescent females than in males ($P = 0.04$, Two-sample t-test) and input resistance was lower in female patients in the late childhood group ($P = 0.03$, Mann-Whitney test) (**Supplementary Figure 18**). We found differences when comparing the AP characteristics in AP half-width cells from male patient showed higher values in early childhood ($P = 0.036$) and adolescence ($P = 0.02$). AP up-stroke velocity was higher in young adulthood females than in males ($P = 0.34$). AP amplitude was significantly higher in male patients

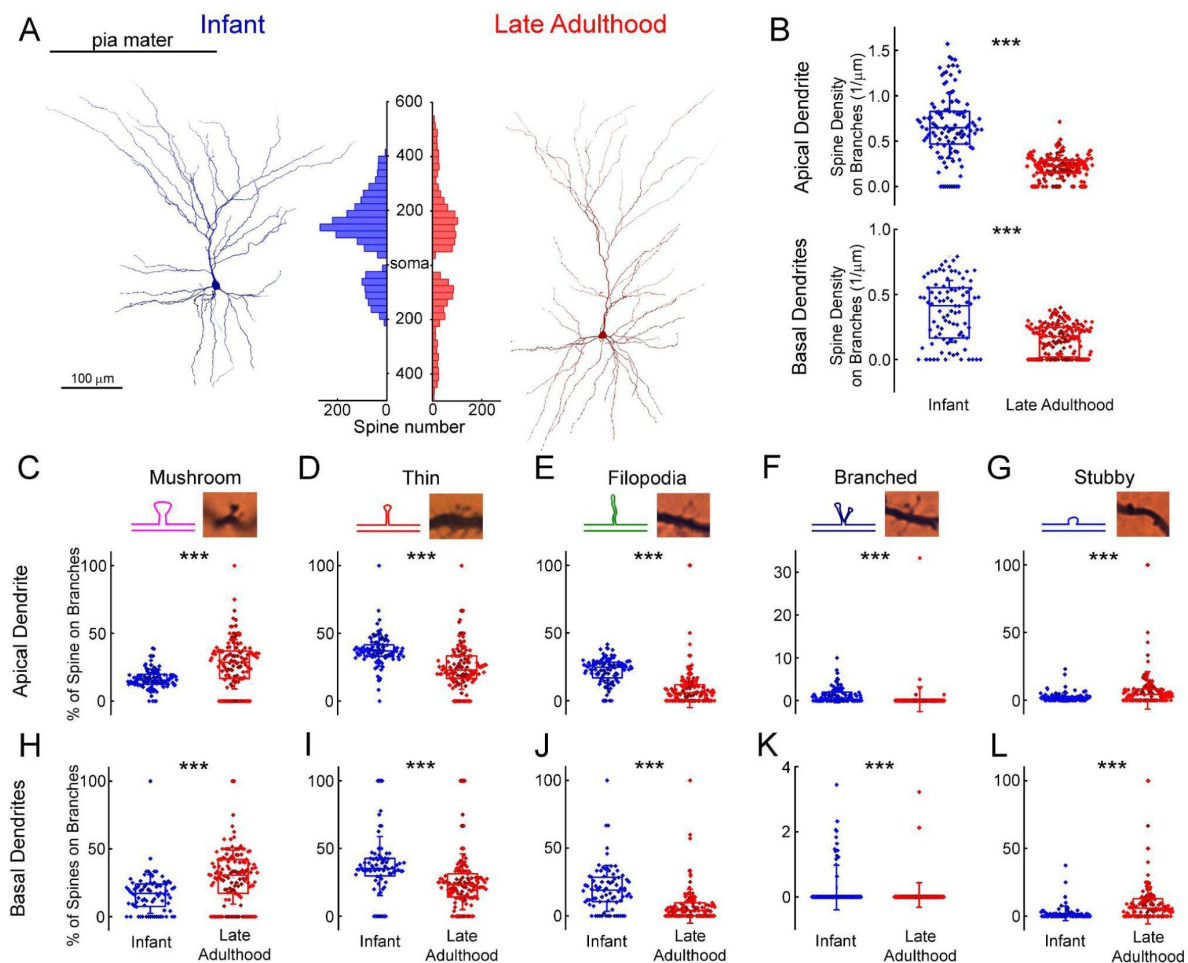


Figure 6.

Comparison of dendritic spine densities in pyramidal cells from infant and late adulthood samples.

(A) Anatomical 3D reconstruction of human L2/3 pyramidal cells from the infant (left), and the late adulthood (right) age groups. The histogram (in the middle) demonstrates the distribution of dendritic spines on the two representative cells according to their distance from the soma (μm) on the apical and basal dendrites.

(B) Boxplots of the average spine densities on the apical (top), and basal (bottom) dendritic branches from the $n = 3$ infant (blue) and $n = 3$ late adulthood (red) L2/3 pyramidal cells. The symbols are color-coded by the 6 individual cells.

(C-G) The plots show the distribution of mushroom (C), thin (D), filopodium (E), branched (F), and stubby (G) dendritic spine types on the apical dendrites of the reconstructed infant ($n = 3$, blue) and late adult ($n = 3$, red) pyramidal cells. Top, schematic illustration and representative images of the examined dendritic spine types. Center, age dependent distribution of spine types. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

(H-L) Same as C-G but on basal dendrites.

in early childhood ($P = 0.003$) and late adulthood ($P = 0.035$) age groups (**Supplementary Figure 19** [↗](#)). Under the comparison of the firing pattern features cells from the infancy showed significant differences in F-I slope ($P = 0.2$, Mann-Whitney test), first AP latency ($P = 0.0096$, Two-sample t-test) and adaptation ($P = 0.002$, Mann-Whitney test). The latency of the first AP was higher in cells from male patient in the young ($P = 0.03$, Mann-Whitney test) and middle ($P = 0.018$, Mann-Whitney test) adulthood cells. The adaptation of the APs in males was higher during adolescence ($P = 0.027$, Mann-Whitney test) but lower in middle adulthood ($P = 0.034$, Mann-Whitney test) (**Supplementary Figure 20** [↗](#)). Regarding the morphological property of the reconstructed cell we found differences in the young adulthood age group between cells from male and female patients in total ($P = 0.03$, Mann-Whitney test) and apical ($P = 0.03$, Mann-Whitney test) dendritic length and the total number of bifurcations ($P = 0.03$, Mann-Whitney test), in the middle adulthood group we found significant difference in the average apical terminal length across males females ($P = 0.03$, Mann-Whitney test) (**Supplementary Figure 21** [↗](#)).

Discussion

In this study, using the whole-cell patch-clamp technique and 3D reconstructions, we have studied the differences of human cortical L2/3 pyramidal cells at various stages of life. We found that during the lifespan the most significant changes take place early in life during the first year in most of the biophysiological characteristics of human pyramidal cells (see **Supplementary Figure 3** [↗](#)). We recorded from $n = 457$ human cortical excitatory pyramidal cells from the supragranular layer from birth to 85 years. Most differences in sub- and suprathreshold features were found in the youngest age groups. There were particular differences in resting membrane potential, input resistance, time constant, rheobasic current, AP halfwidth and AP upstroke velocity. Other age groups that differed the most from other age groups were the oldest (60 - 85 years) with modest differences in sag ratios. In our morphological analysis, we found no significant changes in the overall apical and basal dendritic dimensions across different ages. When evaluating the number of spines in two anatomical reconstructions (from the infant and late adulthood groups) we found a higher overall spine density in the infant than in the elder sample consisting of mainly branched and thin spines and filopodia. In contrast, stubby and mushroom-shaped spines were more prevalent in the older pyramidal cell.

The nervous system experiences a multitude of plastic changes throughout an individual's lifetime. These changes extend from the early stages of development and continue through the gradual degenerative processes that come with old age. Although precise intrinsic cellular modifications linked to aging remain somewhat elusive, there is an observable morphological transformation during early development. As the nervous system matures, dendritic branching (Kasper et al. 1994 [↗](#), McAllister 2000 [↗](#)) and cell body size increases (Zhang 2004). Also, learning and experience increase the extent of dendritic branching (Holloway 1966 [↗](#), Greenough and Volkmar 1973 [↗](#)) and dendritic spines (Globus 1973). An extensive body of research has shown that synaptic plasticity induces formation of new dendritic spines and the enlargement of existing spines (Kastellakis 2023). These modifications may affect the input-output relation of a neuron as model studies suggest that dendritic geometry influences electrical and firing properties (Mainen and Sejnowski 1996 [↗](#), van Elburg and van Ooyen 2010 [↗](#), Eyal 2014, Vetter et al 2001 [↗](#)). Furthermore, in parallel to the changes in morphological complexity there is ample experimental evidence that the maturation of ion channels and the change in increasing channel density in the neuronal membrane also play a role in influencing the electrical properties of neurons (Huguenard et al. 1988 [↗](#), Connors 1994 [↗](#)). These findings point to a close causal relationship between structure and electrophysiological properties at cellular level.

Lifespan changes in electrophysiological properties

The intrinsic electrophysiological properties of a neuron are determined by several factors: the resistivity of the cytoplasm and membrane, the membrane capacitance, and the shape of the soma and dendrites. The basic passive parameters we recorded, such as the resting membrane potential, the input resistance, and the membrane time constant show a dramatical change in the first year. This change is consistent with data found in previous studies on neocortical pyramidal cells from rodent models (Kasper 1994, Kroon 2019, Picken Bahrey and Moody 2003 [↗](#), Zhang 2004, Zhu 2000 [↗](#), McCormick 1987) and on xenotransplanted human cortical neurons (Linaro 2019). We found a progressive shift of the resting membrane potential from -60 mV to -68 mV in the first part of the lifespan (<40 years), which could be due to the age-dependent change in ion channel composition (Picken Bahrey and Moody 2003 [↗](#)). The input resistance and time constant showed a sudden and sharp decrease between the first and second age groups, indicating the most dramatic changes in the infant brain followed by a generalized and slower decrease. As pyramidal cells mature, the size and volume of the cell body increase (Petanjek 2019, Zhu 2000 [↗](#)) which correlates with the input resistance, meaning that a small membrane area has a higher input resistance (Luhmann 2000). In addition to the increase in cell surface area, the increase in the expression of certain potassium leak channels, e.g. from the two-pore domain potassium channel (KCNK) family, can also influence the input resistance during cell maturation (Aller 2008, Goldstein 2001). Similar reasons can lead to a decrease in the membrane time constant. The membrane time constant is the product of the specific input resistance and the specific membrane capacitance. Assuming constant membrane capacitance ($\sim 1 \text{ pF/cm}^2$, Oláh et al 2024 [↗](#), Beaulieu-Laroche 2018), the decrease is likely also due to a change in specific membrane resistance. The result of developmental changes in membrane passive parameters during early development improves spike timing precision and temporal responsiveness in the cortical microcircuit (Oswald and Reyes 2011 [↗](#), Doischer et al. 2008 [↗](#)). However, in our human samples at later ages (>40 years) that we associate with the aging brain, the previous trends of changes in intrinsic characteristics are reversed. Input resistance and time constant increase, which may be associated with the decline in KCNK expression in the aging brain (Erraji-Benchenkroun 2005). These membrane parameters are also generally higher in studies in rhesus monkeys (Moore 2023), except for the resting membrane potential, which is not different from that of young cortical L2/3 (Chang 2005) or L5 pyramidal cells (Luebke 2007) in the brain. However, in our samples, we found a positive shift in the resting potential between the middle- and old-age groups by a few millivolts. It should be noted that this discrepancy may be due to the fact that the age groups in our work are not the same as the works mentioned.

Changes in resting membrane potential and input resistance will add up to produce a rising and then falling curve of the averaged rheobasic current, with the value three times higher in the adolescent group than in the youngest group and 1.5 times higher than in late adulthood. Our results on increased rheobase during early life are consistent with previous studies in rodent cortical L3 and 5 pyramidal cells (Kroon 2019, Perez-García 2021, Popescu 2021) and with findings regarding aging in rhesus monkey cortical neurons from rhesus monkeys (Moore 2023).

When we assessed the voltage sag ratio with age we found a consistent increase that peaked in late adulthood. The voltage sag that occurs during membrane hyperpolarization is the result of activation of the hyperpolarization-activated cyclic nucleotide-gated (HCN) current, also known as the h-current. Similar to our findings the age-dependent increase in sag deflection has been observed in human cortical pyramidal cells (Guet-McCreight 2023, Kalmbach 2018) which is consistent with studies by Wang et al. (2011 [↗](#), 2007 [↗](#)) that have linked inhibited HCN activation to working memory deficits in the elderly.

The electrical output of neurons is driven by the composition of neuronal molecules and ion channels whose expressions and distributions change most dynamically in two periods, early development, and the aging brain. This dynamic essentially produced an inverted U-shaped curve

in the distribution of the active electrical properties over the lifespan in our dataset, an increase in the first year, a relatively stable middle phase, and then a return in older age groups. The origin of these changes is mainly due to changes in the distribution of sodium and potassium channels. During development, there is evidence that the number of voltage-gated Na⁺ channels increases in rodents (Huguenard et al. 1988 [↗](#), Picken Bahrey and Moody 2003 [↗](#)) and in humans (Erraji-Benchekroun 2005), which may accelerate the kinetics of the action potential (up-stroke) and, similar to our results, increase the amplitude of the action potential (Perez-García 2021, Kroon 2019, Zhang 2004, McCormick 1987, Etherington 2011). During aging, however, regulation is disrupted by the change in channel expression, which can lead to altered conduction rate of ionic currents. In brain senescence, the amplitude of the action potential and a slowing of the action potential (Luebke 2007) have been shown to be similar to our data, which may be caused by the decreased expression of voltage-gated Na⁺ channels (Erraji-Benchekroun 2005).

We examined the firing patterns of pyramidal cells at different ages by comparing the F-I slope, and we found that the patterns are fairly conserved across ages. In rodent developmental studies, a decrease in F-I slope was found only in the first two weeks after birth (Zhang 2004, McCormick 1987), which time window is not represented in our data. The latency of the first spike is influenced by the time-constant and specific ion channels, like the transient (or A-type) K⁺ current and the transient Ca²⁺ current (or T-type) (Molineux 2005). The same ion channels are involved in burst firing at the onset of stimulation. Note that in our sample only ~10 % of pyramidal cells showed burst firing at the beginning of stimulation, but it is known that burst firing is more frequent from layer 3 onwards at depth (Berg 2021). The presence of A-current has been found in immature pyramidal neurons from sensorimotor areas in rodent studies (McCormick 1987), and the density of T-type currents also remains unchanged during the development of the visual cortex (Horibe 2014). It has been shown that the expression of the T-type calcium channel family (CACNG3) decreases during aging (Erraji-Benchekroun 2005). However, it must be mentioned that the differences in the expression of all subtypes of the Kv4 (A-type) and Ca_v3.x (T-type) channel families during brain development and aging have not been systematically described.

Properties of dendritic trees and spine distribution with age

Transcriptomic analyses of the human neocortex show relatively stable expression patterns of genes for dendritic and synaptic development after about 1 year of age (Kang 2011). In early postmortem studies human pyramidal neurons were studied for morphological changes as a function of age. In these studies, using Golgi staining methods, it was found that the dendritic trees of infant layer 2/3 pyramidal cells are well developed (Mrzljak et al. 1990 [↗](#)). In the human prefrontal cortex after birth, the dendritic tree of layer 3 pyramidal neurons reaches its structurally mature form after about 3 months (Petanjek 2008). Thereafter, it is assumed that a smaller increase in dendritic length reaches its final form between about 7.5-12-months (Koenderink 1995, Petanjek 2008). In our study, we observed stable patterns of apical and basal dendrite lengths and dendritic tree complexity across the lifespan. Even between the early postnatal groups (infancy vs. early childhood), no significant difference in dendritic tree size was observed. This can be explained by the influence of various factors, such as the large difference between individual subjects or the subtype specific dendritic morphology of pyramidal cells (Berg et al 2021 [↗](#)) or different maturation curves of neurons from different brain regions (Elston and Fujita 2014 [↗](#)) or cortical layers (Petanjek 2008).

Neurons exhibit a phase of synaptogenesis overproducing synapses lasting months or years in the human cortex (Huttenlocher 1979 [↗](#), Huttenlocher et al. 1982 [↗](#), Mrzljak et al. 1990 [↗](#), Petanjek et al. 2011 [↗](#)), followed by dendritic spine/synapse pruning that is reported to last more than a decade in the cortex (Petanjek et al. 2011 [↗](#), Jacobs 1997, Mavroudis 2015, Benavides-Piccione 2013, Coskren 2015). Our results from comparing two groups: infant and late adult pyramidal cells also show that the overall number of dendritic spines decreases with increasing age. When evaluating the distribution of spine shapes in young and old pyramidal cells, we found spines with larger heads (mushroom shape) in a greater number in the dendrite of old pyramidal cells, which

are considered as mature synapses. The size of the head is an indicator of the size of the postsynaptic density, the number of glutamate receptors, and the strength of the synapse (Bourne and Harris 2008 [DOI](#), Matsuzaki 2001, Nusser 1998). In the developing nervous system, however, the filopodia, thin dendritic protrusions without a head, are the most characteristic type of spines. The density of filopodial spines was higher on the dendrites of infant pyramidal cells. In the older cells, we still found filopodia, which are the silent precursor of active synapses (Vardalaki 2022). The change in the ratio of subtypes from young to old age is well established and is associated with the basis for changes in cognitive function during aging (Luebke 2015, Dumitriu 2010, Jacobs 1997).

The complexity of human brain activity and cognitive abilities increases with development and decreases with aging, resulting in an inverted U-shaped curve across the lifespan (Craik and Bialystok 2006 [DOI](#)). Cognitive functions depends on many age dependent factors, such as the density and specificity of synaptic connections formed by synaptic pruning and plasticity, or the degree of myelination and white matter maturation and there is also an age-related modulation of neurotransmitters, hormones, ion transporters and receptors (Luna 2015). Here we have shown that pyramidal cells become less excitable and temporarily more precise during development by changing their intrinsic functional properties and with aging these changes occur somewhat in opposite directions making the intrinsic parameters change symmetrically. In addition some of the changes are asymmetrical either occur with development or with aging, such as the resting membrane potential which mostly changes in young ages or the ratio of sag, which is shifted most in old age. These changes in the intrinsic properties of cells during the first and last stages of life also contribute to the input-output functions of a neuron and ultimately to the age-related development of cognitive abilities.

Materials and methods

Slice preparation

Experiments were performed according to the Declaration of Helsinki with the approval of the University of Szeged Ethical Committee and Regional Human Investigation Review Board (ref. 75/2014). Prior to surgery, the patients provided written consent for all tissue material. We used human cortical tissue adjacent to the pathological lesion that had to be surgically removed from patients (n = 64 female n = 45 male) as part of the treatment for tumors, hydrocephalus, apoplexy, cysts, and arteriovenous malformation. Anesthesia was induced with intravenous midazolam and fentanyl (0.03 mg/kg, 1–2 µg/kg, respectively). A bolus dose of propofol (1–2 mg/kg) was administered intravenously. The patients received 0.5 mg/kg rocuronium to facilitate endotracheal intubation. The trachea was intubated, and the patient was ventilated with a mixture of O₂-N₂O at a ratio of 1:2. Anesthesia was maintained with sevoflurane at a care volume of 1.2–1.5. During the surgical procedure tissue blocks were removed from parietal (n = 22), temporal (n = 35), frontal (n = 42), and occipital (n = 10) regions, the resected tissue blocks were immediately immersed in ice-cold solution. Slices were cut perpendicular to the pia mater at a thickness of 320 µm with a vibrating blade microtome (Microm HM 650 V) in ice-cold solution (in mM) 75 sucrose, 84 NaCl, 2.5 KCl, 1 NaH₂PO₄, 25 NaHCO₃, 0.5 CaCl₂, 4 MgSO₄, 25 D(+)-glucose, saturated with 95% O₂ and 5% CO₂. The slices were incubated in the same solution for 30 minutes at 36°C following that the solution was changed to (in mM) 130 NaCl, 3.5 KCl, 1 NaH₂PO₄, 24 NaHCO₃, 1 CaCl₂, 3 MgSO₄, 10 D(+)-glucose, saturated with 95% O₂ and 5% CO₂, the slices were kept in it until use.

In vitro electrophysiological recordings

Somatic whole-cell current-clamp recordings were obtained at ~36 °C in solution containing (in mM) 130 NaCl, 3.5 KCl, 1 NaH₂PO₄, 24 NaHCO₃, 3 CaCl₂, 1.5 MgSO₄, 10 D(+)-glucose, from layer 2/3 pyramidal cells visualized by infrared differential interference contrast (DIC) video microscopy equipped with micromanipulators (Luigs and Neumann, 652 Ratingen, Germany) and HEKA EPC 9&10 patch clamp amplifier (HEKA Elektronik GmbH, Lambrecht, Germany). Micropipettes (3–5

MΩ) were filled with intracellular solution containing (in mM) 126 potassium-gluconate, 4 KCl, 4 ATP-Mg, 0.3 GTP-Na₂, 10 HEPES, 10 phosphocreatine, and 8 biocytin (pH 7.20; 300 mOsm). After whole-cell configuration was obtained stepwise currents were injected to measure the evoked sub- and suprathreshold membrane potential properties. For the analysis of the electrophysiological recordings $n = 457$ recordings with a series resistance (R_s) of 24.93 ± 11.18 MΩ (max: 63.77 MΩ) were used. For the analysis of fast parameters related to the action potential (AP half-width, AP upstroke velocity, AP amplitude and rheobase), higher quality requirements were set and cells with $R_s > 30$ MΩ were excluded. This reduced the data set to $n = 331$ cells with R_s 19.42 ± 6.2 MΩ.

Data analysis

Electrophysiological features were measured from voltage responses elicited by 800 ms long current steps increasing by 20 pA from -100 pA. We analyzed the electrophysiological data with Fitmaster software (HEKA Elektronik GmbH, Lambrecht), and custom MATLAB (The Math Works, Inc.) scripts.

Analysis of morphological features was made by NeuroExplorer software (MBF Bioscience, Williston, VT, USA) and Origin 9 (OriginLab, Northampton, MA).

Statistics

We used custom-written R scripts (R 4.1.2) and the `gamm` function from the `mgcv` R package (`mgcv` 1.8.38). Data presented as the mean $\pm$ s.d. Normality was tested with the Lilliefors test, for statistical analysis, ANOVA with posthoc Bonferroni test, Kurskal-Wallis with posthoc Dunn test, for pairwise comparison two-sample t-test or Mann-Whitney test was used. Differences were accepted as significant if $p < 0.05$. The data are shown on boxplots, boxes indicate 25th, 50th (median), and 75th percentiles, rectangle represents the mean value, and whiskers indicate s.d.

Histology and reconstruction

Slices were fixed in a fixative of 4% paraformaldehyde, 15% picric acid, and 1.25% glutaraldehyde in 0.1 M phosphate (PB, pH = 7.4) for at least 12 hours after electrophysiological recording. After multiple washes in 0.1 M PB, slices were cryoprotected in 10% then 20% sucrose solution in 0.1 M PB. The slices were frozen in liquid nitrogen and then thawed in PB. Slices were embedded in 10% gelatin and further sectioned into 70 μ m thick sections. Sections were incubated in a solution containing conjugated avidin-biotin horseradish peroxidase (ABC; 1:100; Vector Labs) in Tris-buffered saline (TBS, pH = 7.4) overnight at 4°C. The enzyme reaction became visible by using 0.05% 3'3'-diaminobenzidine tetrahydrochloride as a chromogen and 0.01% H₂O₂ as an oxidant. Sections were post-fixed with 1% OsO₄ in 0.1 M PB. Following several washes with distilled water, sections were stained in 1% uranyl acetate, and dehydrated in an ascending series of ethanol. The sections were infiltrated with epoxy resin (Durcupan, Sigma-Aldrich) overnight and embedded on glass slides. After the electrophysiologically recorded cells have been visualized by DAB staining, 3D light microscopic reconstructions were carried out using the NeuroLucida system (MBF Bioscience, Williston, VT, USA) with a 100 $\times$ objective. The length of terminal segments was measured between the terminal tip of the dendrites and the last branching point before the terminal tip. Dendritic spine density on the dendritic branches was calculated as spine/ μ m between two bifurcations. The percentage of spines on branches was calculated as the percentage of the given spine type from all the types of spines on that branch.

Supplementary figures and tables

Supplementary table 1.

Patient metadata

Table showing the patients age, gender, brain region (F - frontal, T - temporal, P - parietal, O - occipital, PO - parieto-occipital, FT - fronto-temporal, TO - temporo-occipital, FP - fronto-parietal), hemisphere and the surgical procedure.

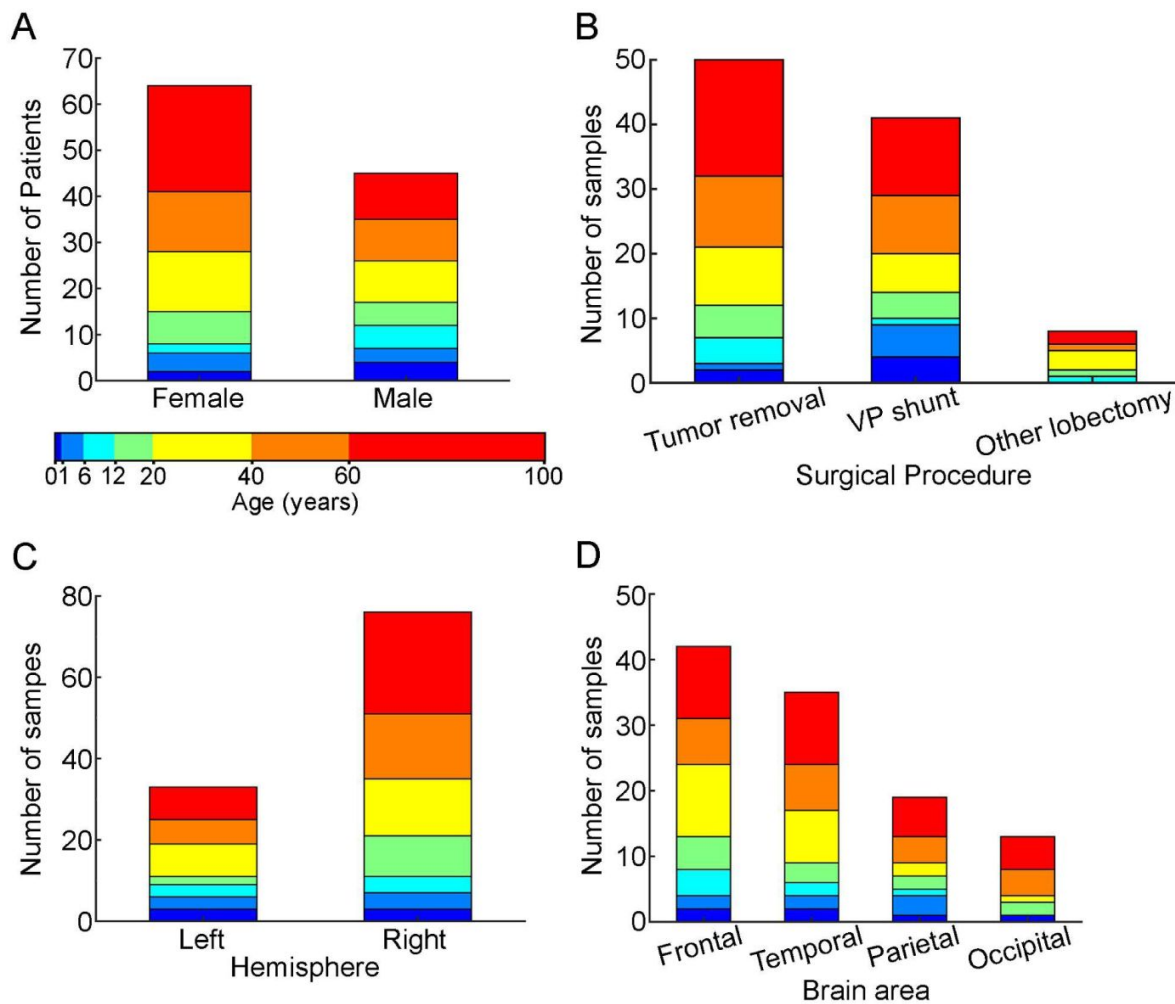
Age (years)	Sex	Brain region	Hemisphere	Surgical procedure	Age (years)	Sex	Brain region	Hemisphere	Surgical procedure
0.66	female	T	right	tumor removal	45	female	F	right	tumor removal
0.08	male	T	right	shunt	41	male	T	right	shunt
0.28	male	O	left	shunt	53	male	T	right	tumor removal
0.1	female	F	left	tumor removal	55	female	T	left	tumor removal
0.23	male	P	right	shunt	43	male	T	right	tumor removal
0.67	male	F	left	shunt	51	female	F	right	other
2	male	F	left	shunt	46	female	P	right	shunt
1	female	F	right	shunt	58	male	T	left	tumor removal
4	male	T	left	tumor removal	57	female	T	right	tumor removal
5	female	P	right	tumor removal	58	female	T	left	tumor removal
2	male	P	right	shunt	47	female	F	right	tumor removal
2	female	P	right	shunt	51	male	O	left	shunt
1	female	TO	left	shunt	43	male	F	right	shunt
7	male	F	right	tumor removal	59	female	P	right	shunt
7	male	P	right	tumor removal	57	female	O	right	shunt
10	male	F	left	tumor removal	43	female	O	right	shunt
10	female	F	left	tumor removal	56	female	F	left	shunt
11	female	F	right	tumor removal	51	male	FT	left	tumor removal
11	male	T	left	shunt	49	female	F	right	tumor removal
10	male	T	right	other	40	female	P	right	tumor removal
13	female	F	right	tumor removal	49	male	O	right	tumor removal
16	male	PO	right	tumor removal	70	male	T	left	tumor removal
18	male	F	right	tumor removal	72	female	T	right	tumor removal
18	female	T	left	tumor removal	81	female	O	right	shunt
19	male	O	right	shunt	63	male	F	right	tumor removal
19	female	F	right	tumor removal	65	male	T	right	tumor removal
19	male	P	right	shunt	69	female	P	left	other
16	male	T	right	tumor removal	70	female	P	right	shunt
13	female	T	right	other	77	male	P	right	shunt
15	female	F	right	shunt	79	female	P	right	shunt
15	female	O	left	shunt	63	female	T	right	tumor removal
19	female	F	right	tumor removal	64	male	T	left	tumor removal
28	female	F	right	tumor removal	69	female	O	right	shunt
27	female	F	right	other	66	female	F	right	tumor removal
20	male	T	right	tumor removal	75	female	O	left	shunt
30	female	F	right	shunt	78	female	T	right	shunt
25	female	O	right	shunt	60	female	T	right	tumor removal
29	female	T	left	other	60	female	F	right	tumor removal
25	female	FT	left	tumor removal	62	female	F	right	shunt
23	female	T	right	other	62	female	T	right	tumor removal
26	female	F	right	shunt	62	female	P	left	tumor removal
30	male	F	left	tumor removal	63	female	F	right	tumor removal
21	female	F	right	shunt	63	female	O	right	tumor removal
35	male	T	right	tumor removal	63	female	F	left	tumor removal
35	female	TO	left	tumor removal	63	female	F	right	shunt
39	female	F	left	tumor removal	64	male	F	right	tumor removal
36	male	T	left	tumor removal	76	male	F	right	tumor removal
32	male	T	right	tumor removal	85	male	FT	right	shunt
33	male	F	right	shunt	85	female	O	left	shunt
34	male	P	left	tumor removal	82	female	T	right	other
20	male	F	right	shunt	73	male	T	right	tumor removal
30	female	F	right	tumor removal	68	female	T	right	tumor removal
26	female	P	left	tumor removal	66	female	P	right	shunt
20	male	T	right	tumor removal	64	male	FP	left	tumor removal
45	male	P	right	shunt					

	Infant					Late adulthood				
	#1 cell	#2 cell	#3 cell	Mean	SD	#1 cell	#2 cell	#3 cell	Mean	SD
Total spine number	3039	5883	4443	4455	1422.04	1909	2977	1553	2146.33	741.07
Apical spine number	2352	3256	3568	3058.67	631.56	1237	1282	849	1122.67	238.07
Basal spine number	687	2627	875	1396.33	1069.93	672	1695	704	1023.67	581.61
Mushroom	477	970	762	736.33	247.5	629	1085	407	707	345.66
Thin	1055	2157	1666	1626	552.09	455	680	403	512.67	147.23
Filopodia	732	1431	936	1033	359.45	223	154	105	160.67	59.28
Branched	31	62	53	48.67	15.95	5	1	2	2.67	2.08
Stubby	67	99	84	83.33	16.01	83	247	134	154.67	83.93
Not classified	677	1164	942	927.67	243.82	514	810	502	608.67	174.46

Supplementary table 2.

Number of dendritic spines on the examined cells.

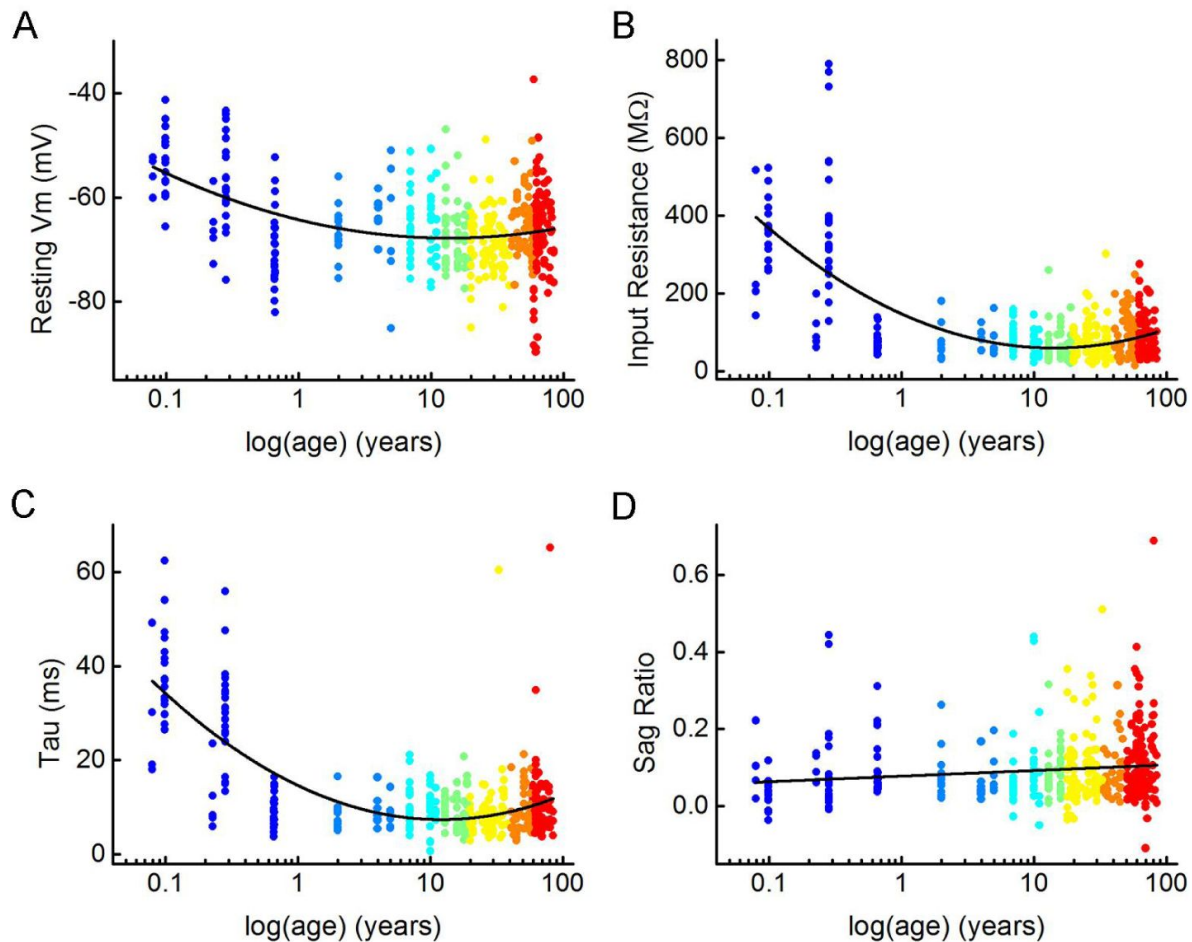
Table showing the overall number of dendritic spines, the total number of spines on the apical and basal dendritic arbour and the number of dendritic spines from different phenotypic subtypes of the individual pyramidal cells (n = 3 infant and n = 3 late adulthood).



Supplementary Figure 1.

Patient metadata

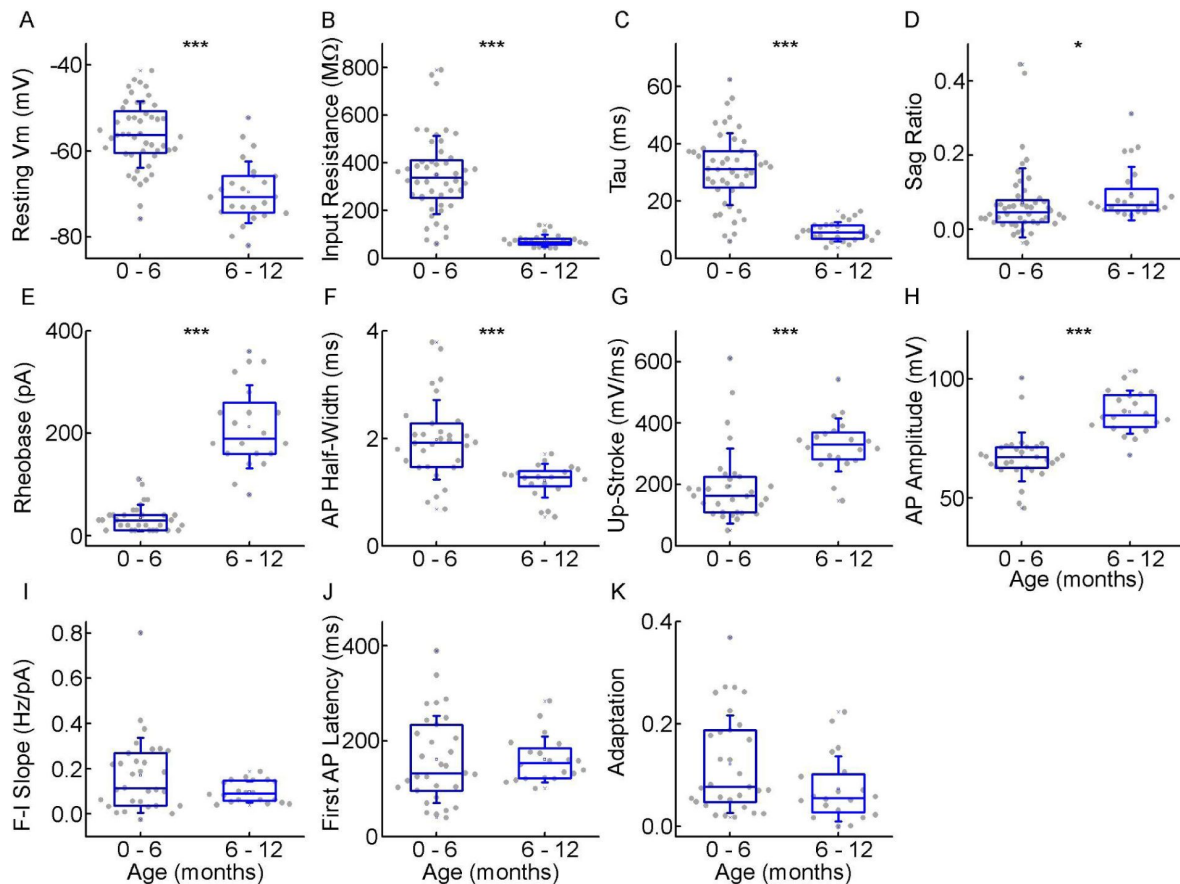
Distribution of patients by gender (female: infant $n = 2$, early childhood $n = 4$, late childhood $n = 2$, adolescence $n = 7$, young adulthood $n = 13$, middle adulthood $n = 13$, late adulthood $n = 23$, male: infant $n = 4$, early childhood $n = 3$, late childhood $n = 5$, adolescence $n = 5$, young adulthood $n = 9$, middle adulthood $n = 9$, late adulthood $n = 10$) (A), surgical procedure (tumor removal: infant $n = 2$, early childhood $n = 2$, late childhood $n = 5$, adolescence $n = 7$, young adulthood $n = 13$, middle adulthood $n = 12$, late adulthood $n = 19$, VP (ventriculoperitoneal) shunt: infant $n = 4$, early childhood $n = 5$, late childhood $n = 1$, adolescence $n = 4$, young adulthood $n = 6$, middle adulthood $n = 9$, late adulthood $n = 12$, other: infant $n = 0$, early childhood $n = 0$, late childhood $n = 1$, adolescence $n = 1$, young adulthood $n = 3$, middle adulthood $n = 1$, late adulthood $n = 2$) (B), hemisphere (left: infant $n = 3$, early childhood $n = 3$, late childhood $n = 3$, adolescence $n = 2$, young adulthood $n = 8$, middle adulthood $n = 6$, late adulthood $n = 8$, right: infant $n = 3$, early childhood $n = 4$, late childhood $n = 4$, adolescence $n = 10$, young adulthood $n = 14$, middle adulthood $n = 16$, late adulthood $n = 25$) (C) and brain area (frontal: infant $n = 2$, early childhood $n = 2$, late childhood $n = 4$, adolescence $n = 5$, young adulthood $n = 11$, middle adulthood $n = 7$, late adulthood $n = 11$, temporal: infant $n = 2$, early childhood $n = 2$, late childhood $n = 2$, adolescence $n = 3$, young adulthood $n = 8$, middle adulthood $n = 7$, late adulthood $n = 11$, parietal: infant $n = 1$, early childhood $n = 3$, late childhood $n = 1$, adolescence $n = 2$, young adulthood $n = 2$, middle adulthood $n = 4$, late adulthood $n = 6$, occipital: infant $n = 1$, early childhood $n = 0$, late childhood $n = 0$, adolescence $n = 2$, young adulthood $n = 1$, middle adulthood $n = 4$, late adulthood $n = 5$) (D). Stacked columns are colored regarding the age groups shown on the colorbar.



Supplementary Figure 2.

Distribution of subthreshold electrophysiological features with age.

(A-D) Scatter plots showing the passive electrophysiological characteristics: resting membrane potential (A), input resistance (B), tau (C), and sag ratio (D) throughout the lifespan. Dots are colored according to the age groups, age is represented in years on a logarithmic scale.



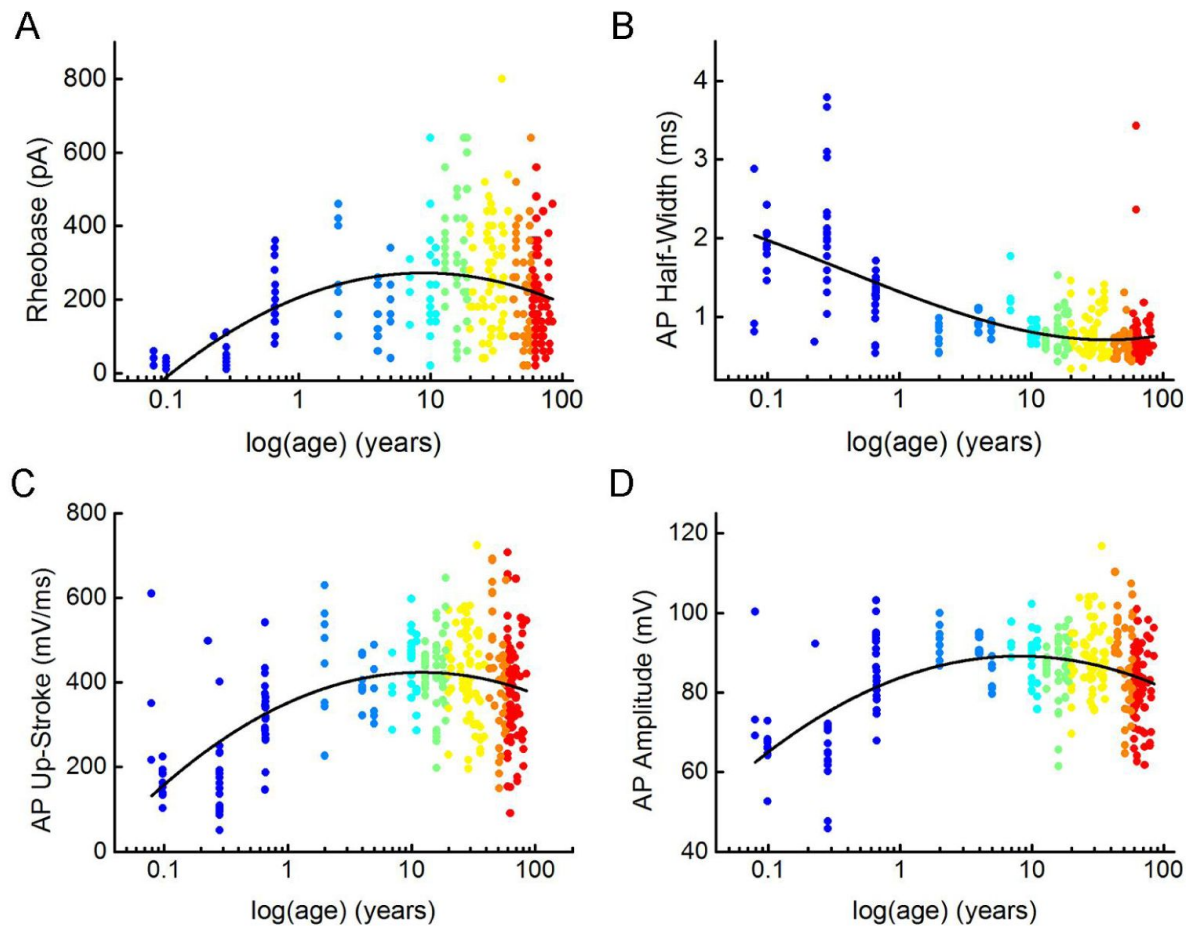
Supplementary Figure 3.

Electrophysiological differences during the first year of life

A-D, Boxplots showing differences in passive properties, resting membrane potential (A), input resistance (B), tau (C) and sag ratio (D) within the infant age group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, two sample t-test (A, B, C) or Mann-Whitney test (D)).

E-H, Differences in the action potential kinetics, Rheobase (E), AP half-width (F), up-stroke (G), and AP amplitude (H) between the cells from the first and the second half of the first year of life. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Mann-Whitney test).

I-K, Deviations in AP firing pattern parameters, F-I slope (H), first AP latency (I), and adaptation (J) during infancy.



Supplementary figure 4.

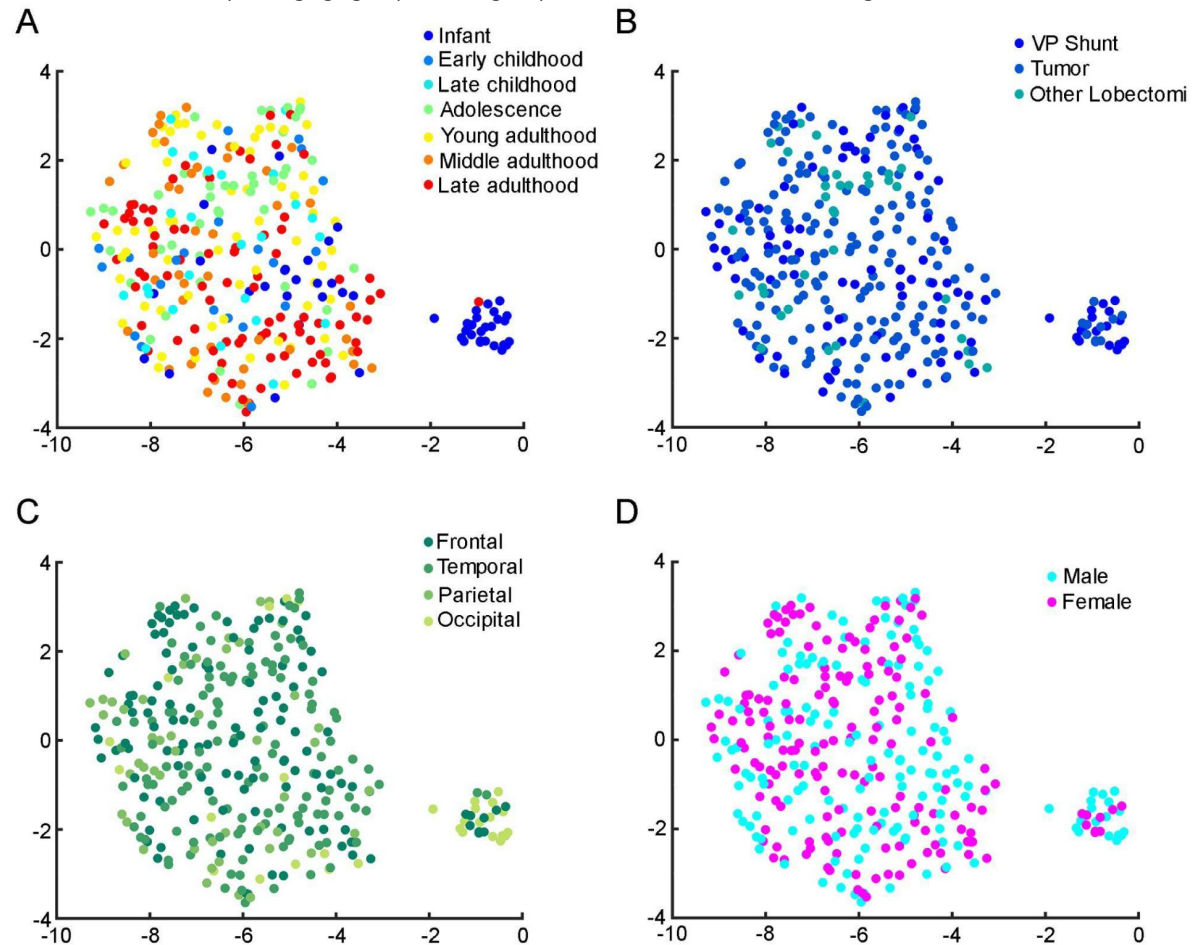
Distribution of suprathreshold electrophysiological features with age.

(A-D) Diagrams show changes through age in rheobase (A), action potential half-width (B), action potential up-stroke (C), and action potential amplitude (D). Dots are colored according to the age groups, age is represented in years on a logarithmic scale.

Supplementary Figure 5.

Relationship between patient metadata and electrophysiology.

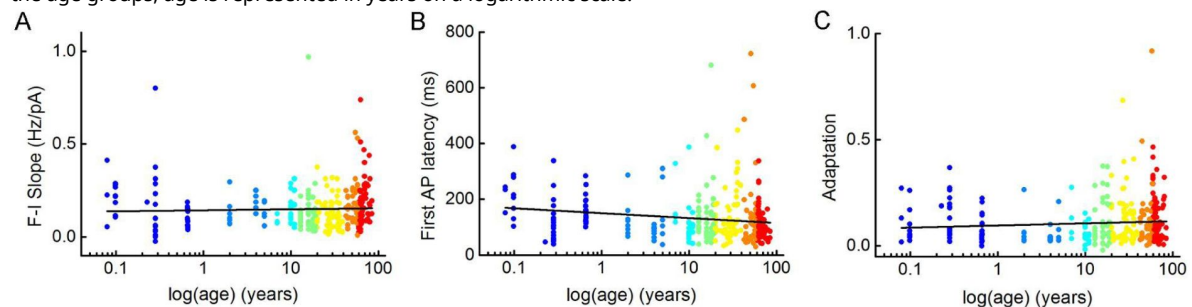
Uniform Manifold Approximation and Projection (UMAP) of 8 electrophysiological properties (resting Vm, input resistance, tau, sag ratio, rheobase, AP half-width, AP up-stroke, and AP amplitude) with data points for 331 cortical L2/3 pyramidal cells, colored with the corresponding age groups (A), surgical procedures (B), brain area (C) and gender (D).

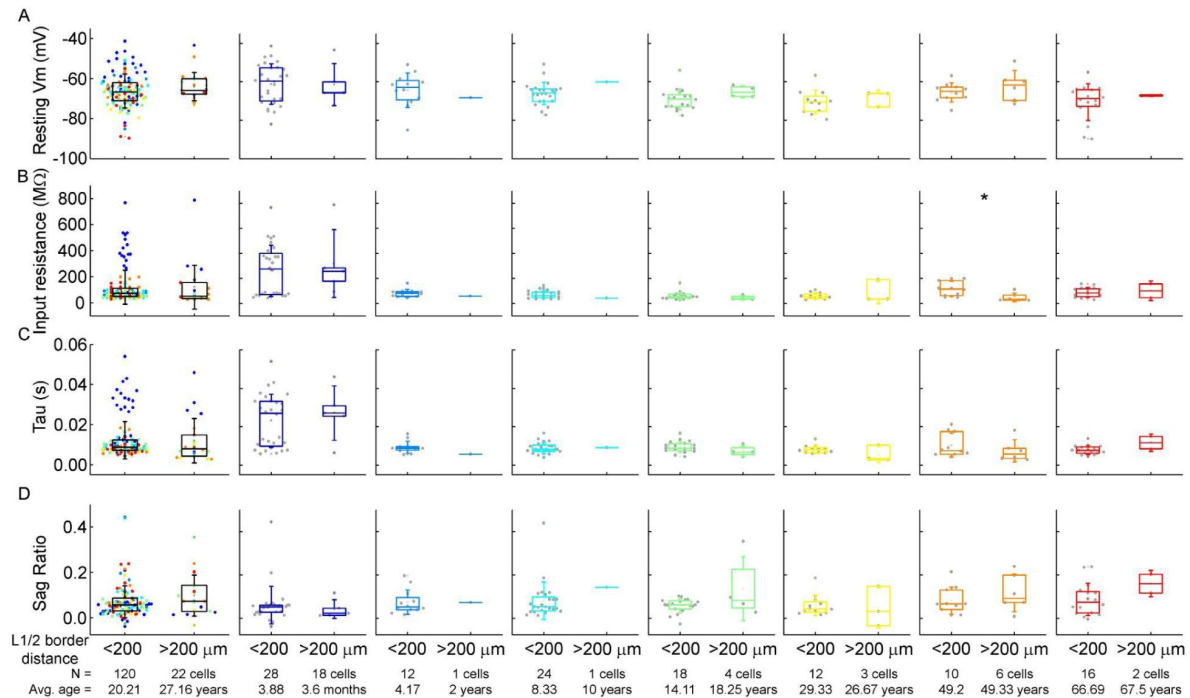


Supplementary Figure 6.

Distribution of firing pattern characteristics with age.

(A-C) Plots show F-I slope (A), first AP latency (B), and adaptation of APs (C) depending on age. Dots are colored according to the age groups, age is represented in years on a logarithmic scale.

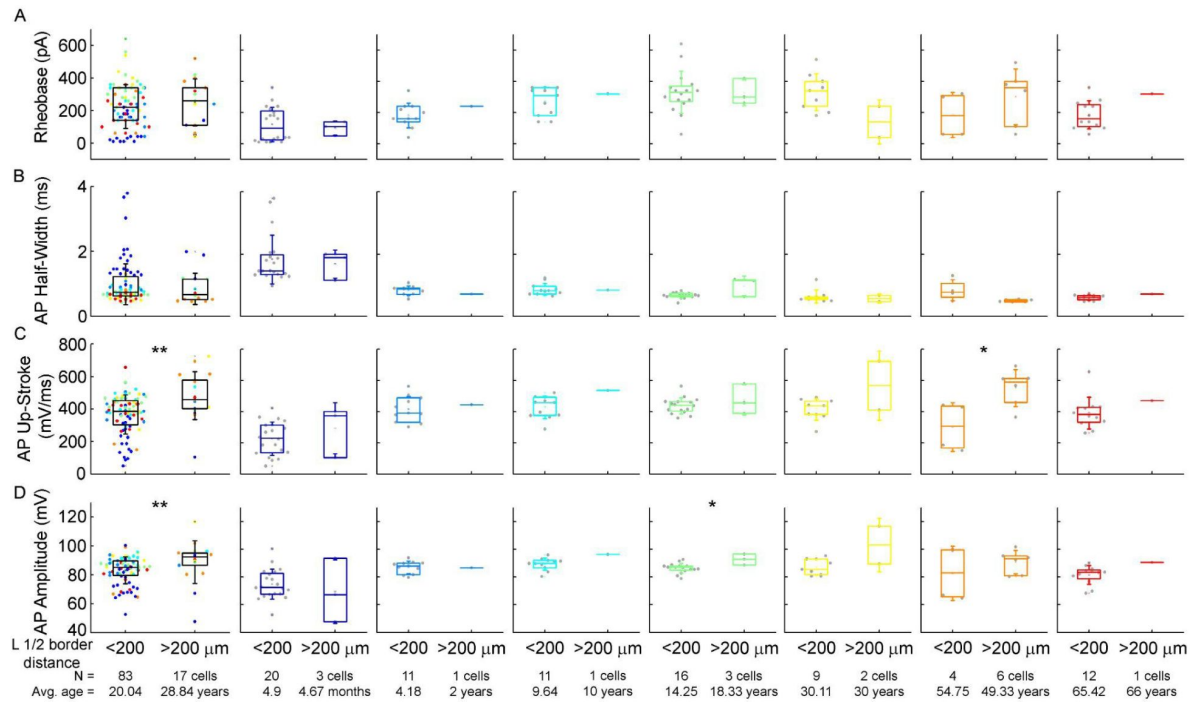




Supplementary Figure 7.

Comparison of the subthreshold properties of the cells as a function of their distance from the layer border.

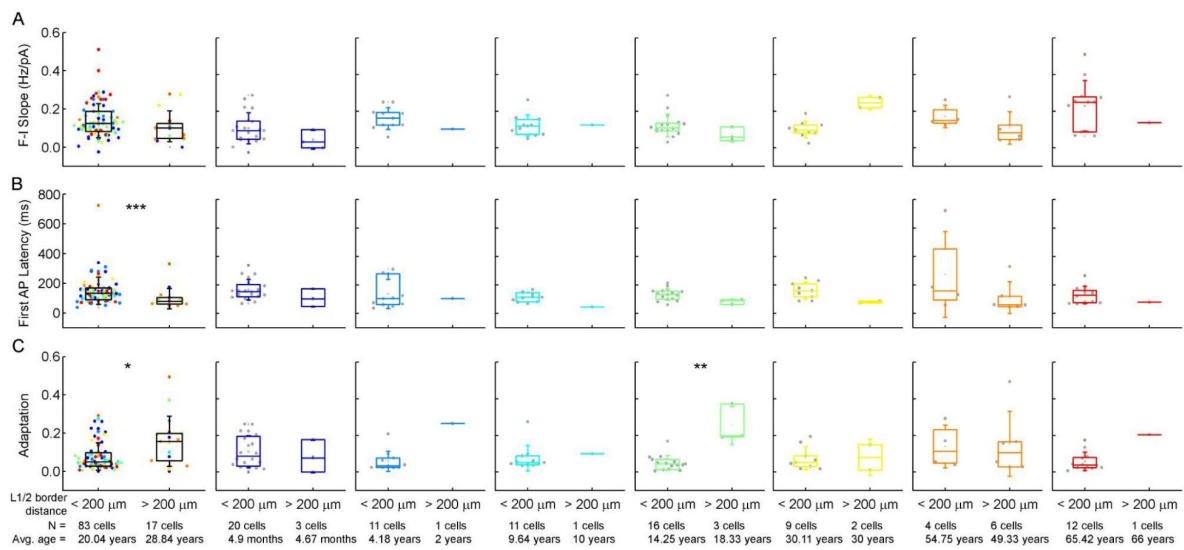
A-D: Box plots show resting membrane potential (A), input resistance (B), tau (C), and sag ratio (D) of the pyramidal cells whose soma is located at a distance greater than and less than 200 μm from the L1 border. From left to right: data from all age groups, infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood and late adulthood groups. Asterisks indicate significance (* $P < 0.05$, Mann-Whitney test).



Supplementary Figure 8.

Comparison of the action potential properties of the cells as a function of their distance from the layer border.

A-C : Boxplots show rheobase (A), action potential half-width (B), action potential upstroke (C) and action potential amplitude (D) of cells whose soma is located at a distance greater than and less than 200 μm from the L1 border. From left to right: data from all age groups, infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood and late adulthood. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$, Mann-Whitney test).

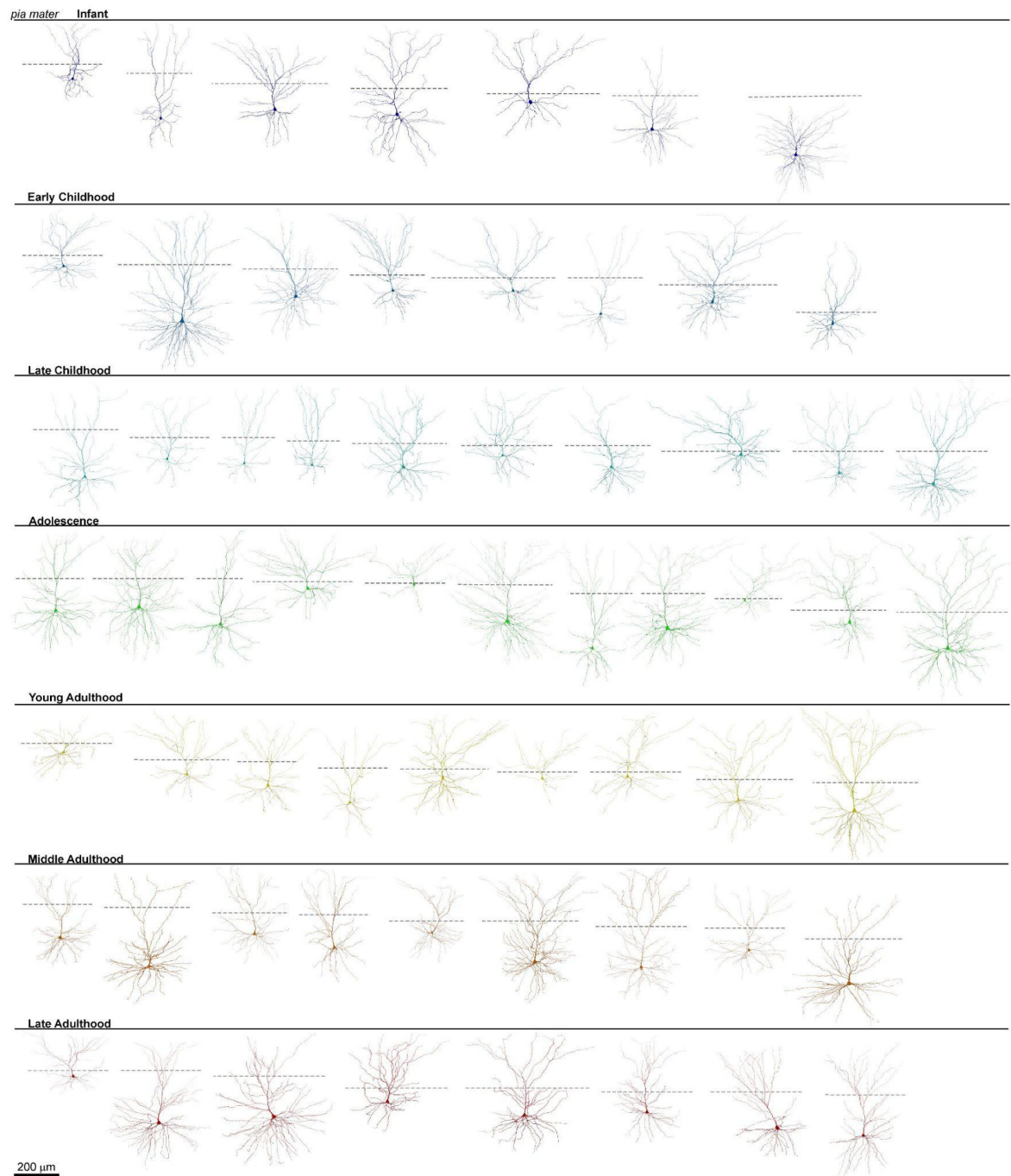


Supplementary Figure 9.

Comparison of the firing pattern characteristics of the cells as a function of their distance from the layer border.

A-C : Boxplots show: F-I slope (A), latency of the first AP (B) and adaptation of APs (C) of cells whose soma is located at a distance greater than and less than 200 μm from the L1 border.

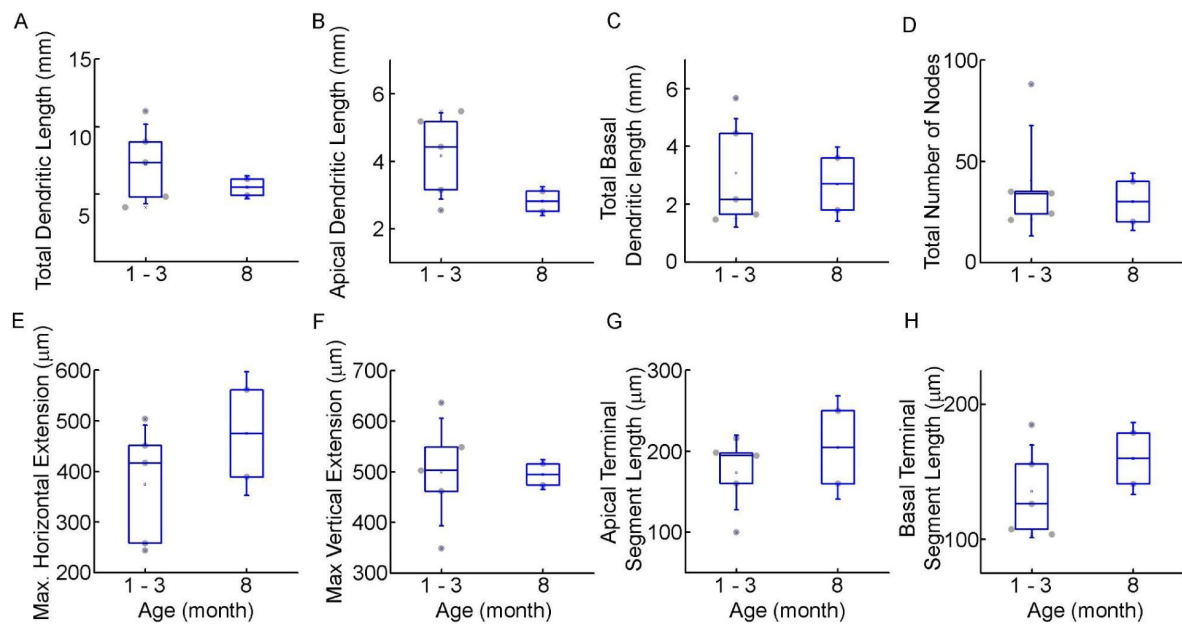
From left to right: data from all ages, infancy, early childhood, late childhood, adolescence, young adulthood, middle adulthood and late adulthood. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$, Mann-Whitney test).



Supplementary Figure 10.

Human cortical L2/3 pyramidal cell dendritic reconstructions.

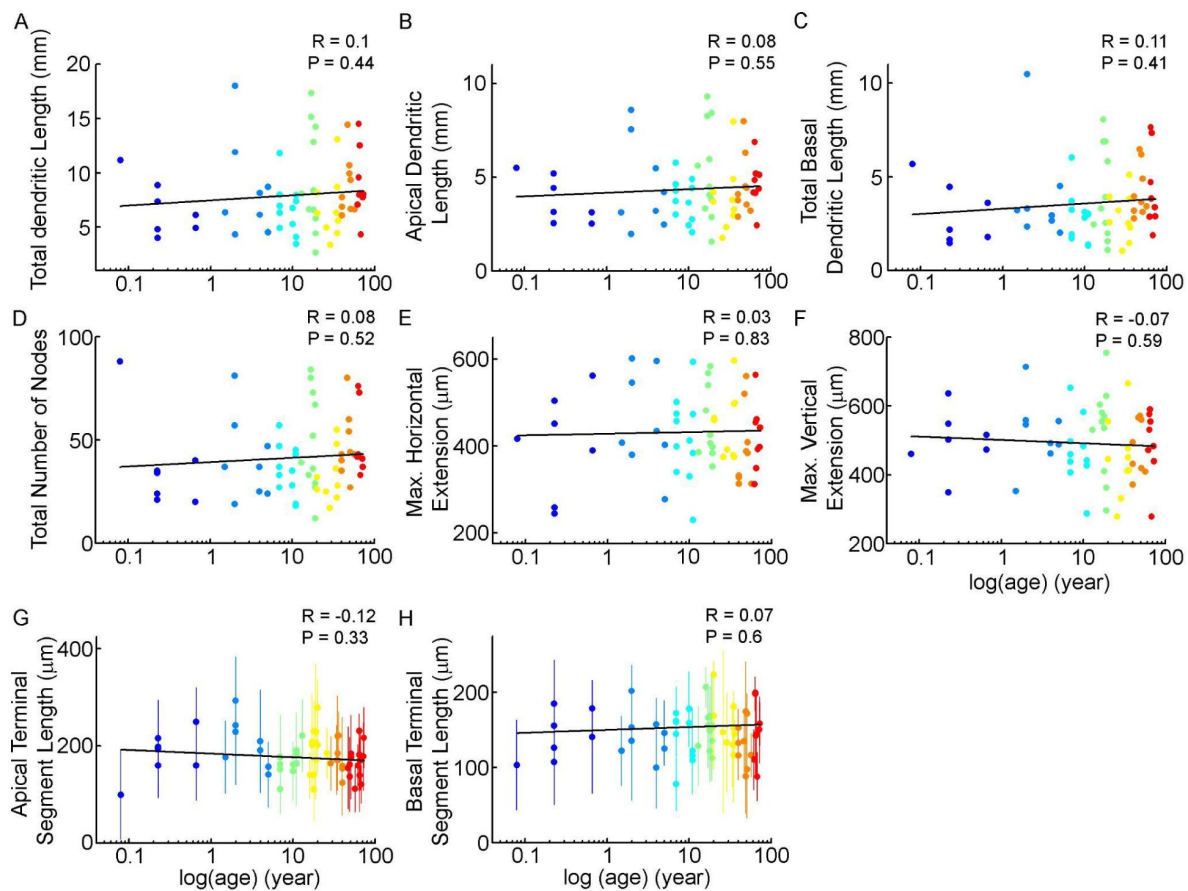
Reconstructions ($n = 63$) of the examined human cortical pyramidal cells, from top to bottom: infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood and late adulthood age groups. Black lines are representing the pia mater, gray dashed lines represent the L1-L2 border.



Supplementary Figure 11.

Morphological comparison of the examined infant cells.

A-H: Boxplots show morphological features: total (A), apical (B), total basal (C) dendritic length, total number of nodes (D), maximal horizontal (E), and vertical (F) extension, average apical (G) and basal (H) terminal dendritic segment from infant patient during the first and second half of the first year of life.

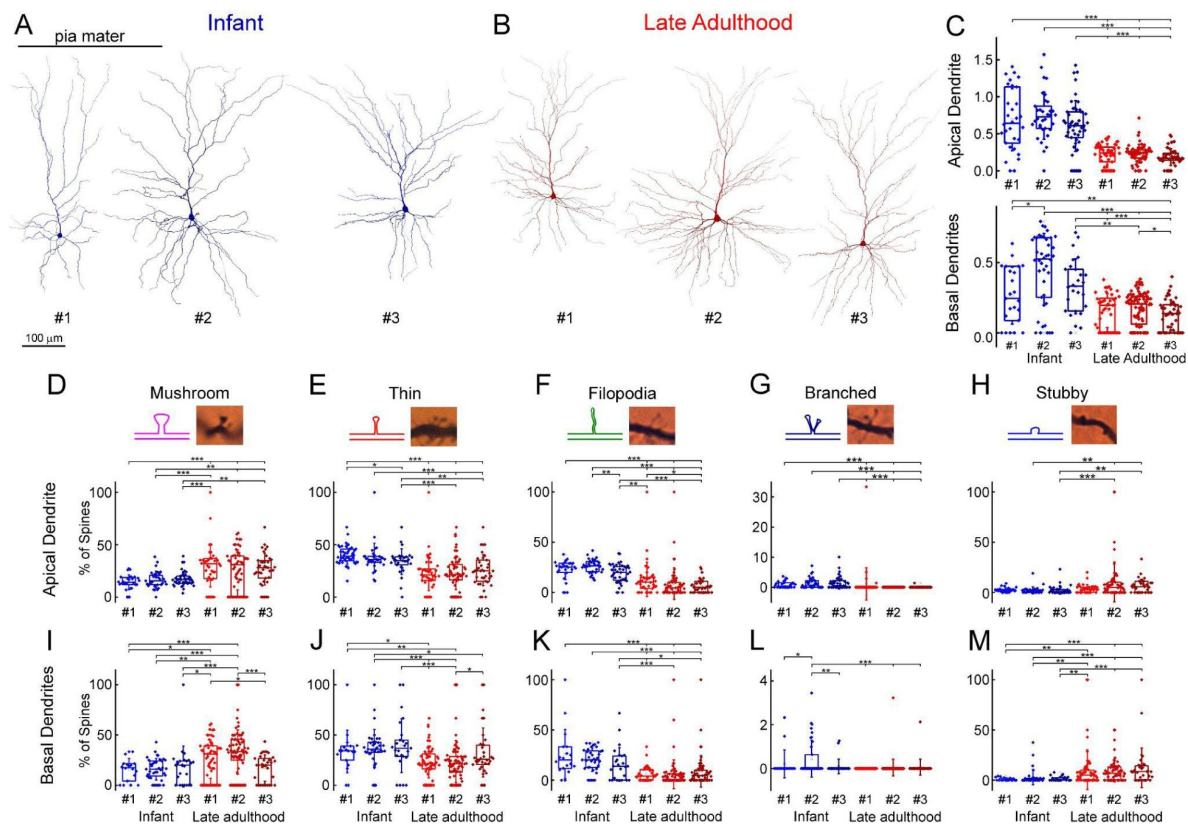


Supplementary Figure 12.

Distribution of morphological features with age

A-F: Scatterplots show the distribution of total (A), apical (B), total basal dendritic length (C), total number of bifurcations (D), maximum horizontal extension (E), maximum vertical extension of the dendritic arborisation (F). The age is represented in years on a logarithmic scale.

G-H: Scatterplots show the average apical (G) and basal (H) terminal segment length of the cells, dots indicate the mean segment length of cells, vertical lines show the standard deviation.



Supplementary Figure 13.

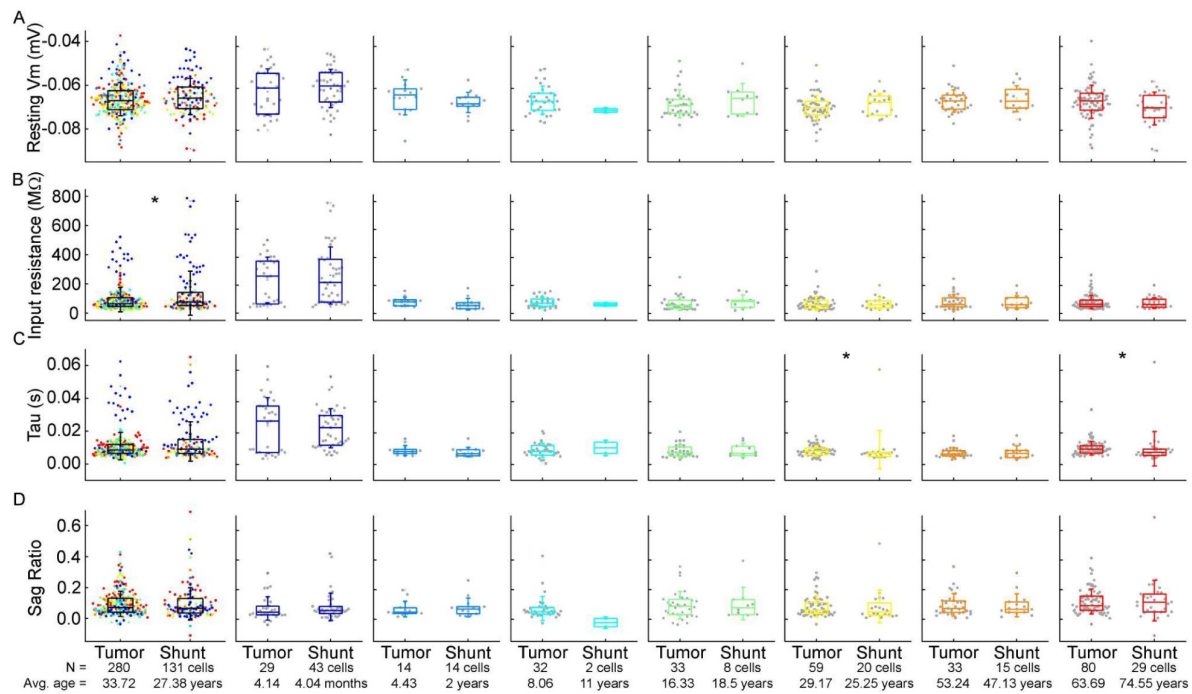
(A) Anatomical 3D reconstructions of the examined $n = 3$ human L2/3 pyramidal cells from the infant age group.

(B) Anatomical reconstructions ($n = 3$) from the late adulthood group.

(C) Boxplots showing the spine density on the six individual cells, the infant pyramidal cell shown with blue, the late adulthood pyramidal cell with red (cells are numbered according to figure A, B), on the apical (top) and the basal (bottom) dendrites. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Kruskal-Wallis test with post-hoc Dunn test).

(D-H) The plots show the distribution of mushroom (D), thin (E), filopodium (F), branched (G), and stubby (H) dendritic spine types on the apical dendrites of the reconstructed infant ($n = 3$, blue) and late adult ($n = 3$, red) pyramidal cells. Top, schematic illustration and representative images representation of the examined dendritic spine types. Bottom, spine distributions on the individual cells that were examined. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Kruskal-Wallis test with post-hoc Dunn test).

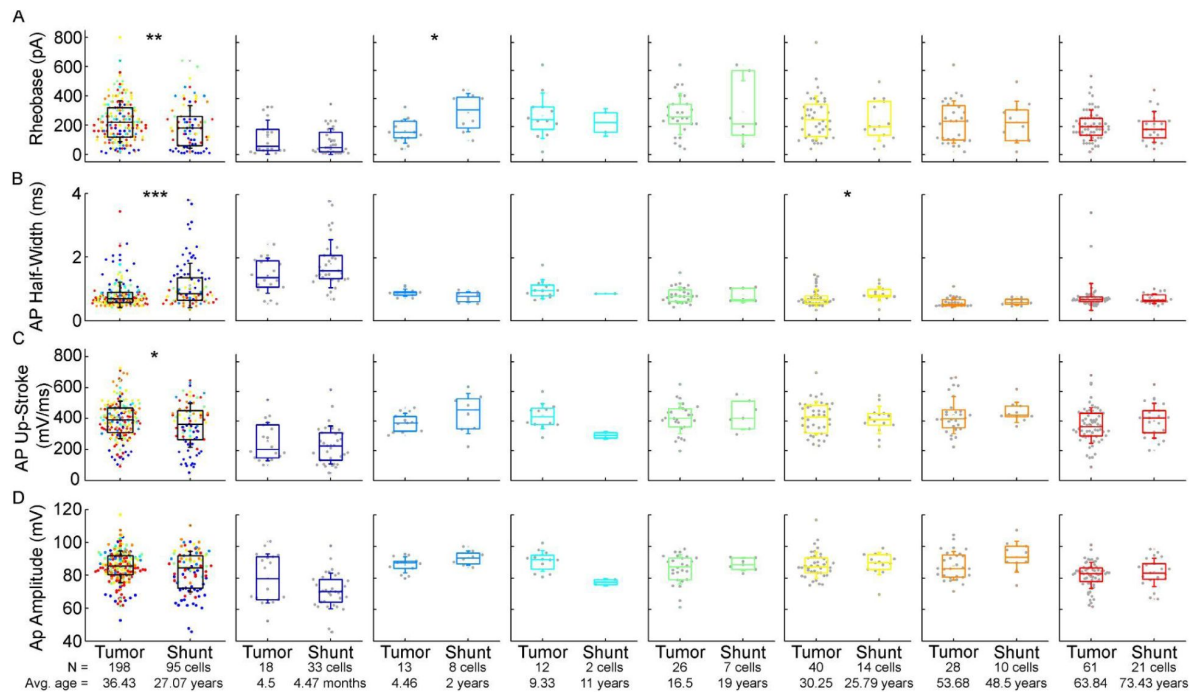
(I-M) Same as D-H but on basal dendrites.



Supplementary Figure 14.

Comparison of subthreshold electrophysiological properties of the examined cells from patients with tumor removal or VP shunt surgical procedures.

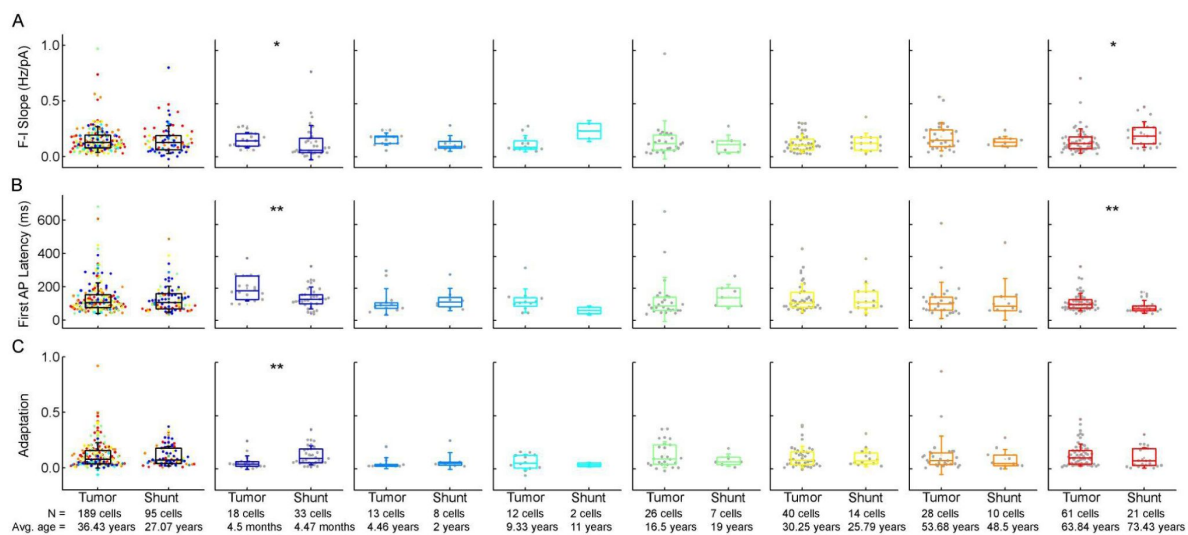
A-D : Box plots show resting membrane potential (A), input resistance (B), tau (C), and sag ratio (D) from patients with tumor removal (tumor) or VP shunt (shunt) surgical procedures. From left to right: data from all age groups, infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood and late adulthood groups. Asterisks indicate significance (* $P < 0.05$, Mann-Whitney test).



Supplementary Figure 15.

Comparison of action potential properties of the examined cells from patients with tumor removal or VP shunt surgical procedures.

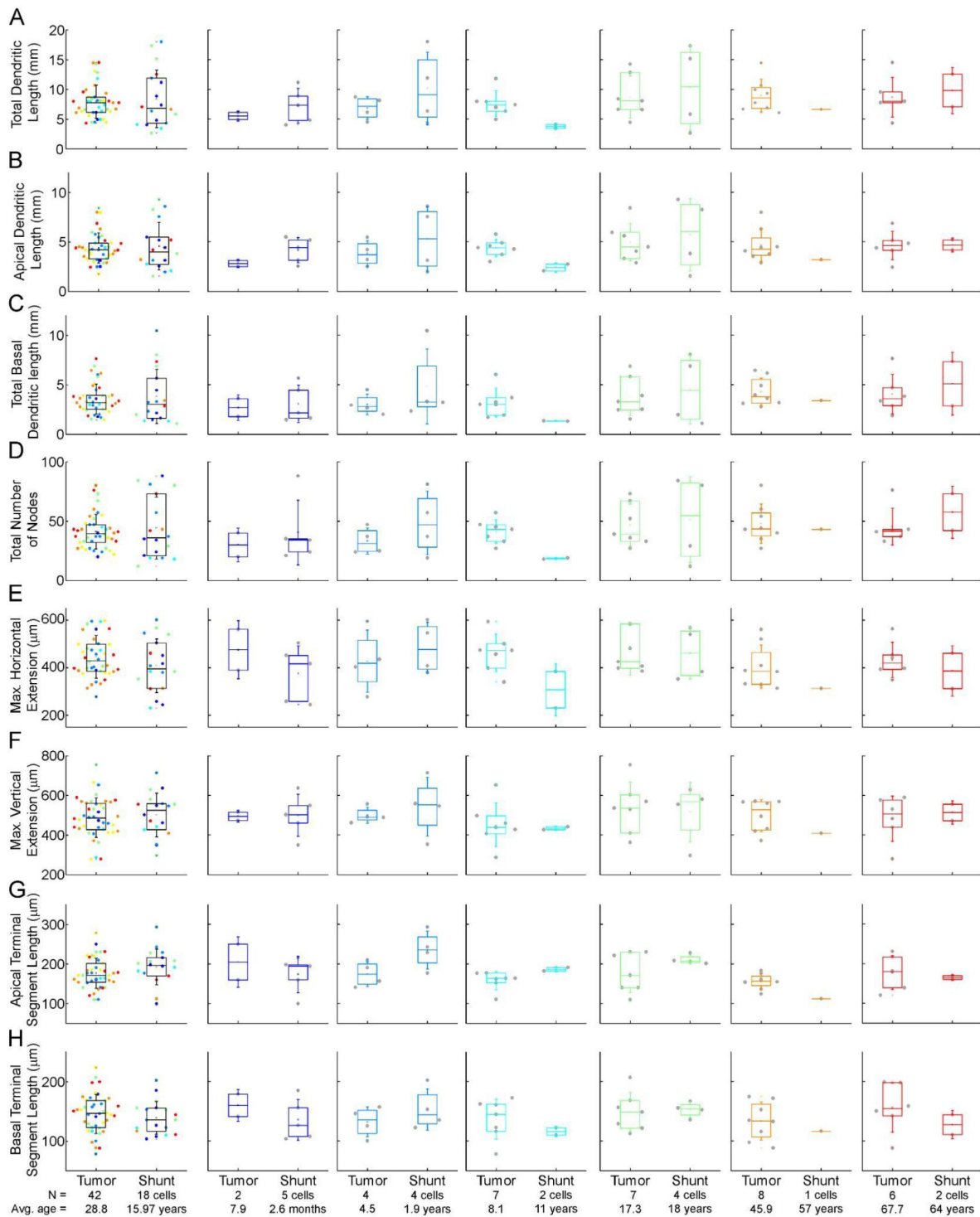
A-D : Boxplots show rheobase (A), action potential half-width mean (B), action potential up-stroke (C) and action potential amplitude (D) from patients with tumor removal (tumor) or VP shunt (shunt) surgical procedures. From left to right: data from all age groups, infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood and late adulthood groups. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Mann-Whitney test).



Supplementary Figure 16.

Comparison of firing pattern characteristics of the examined cells from patients with tumor removal or VP shunt surgical procedures.

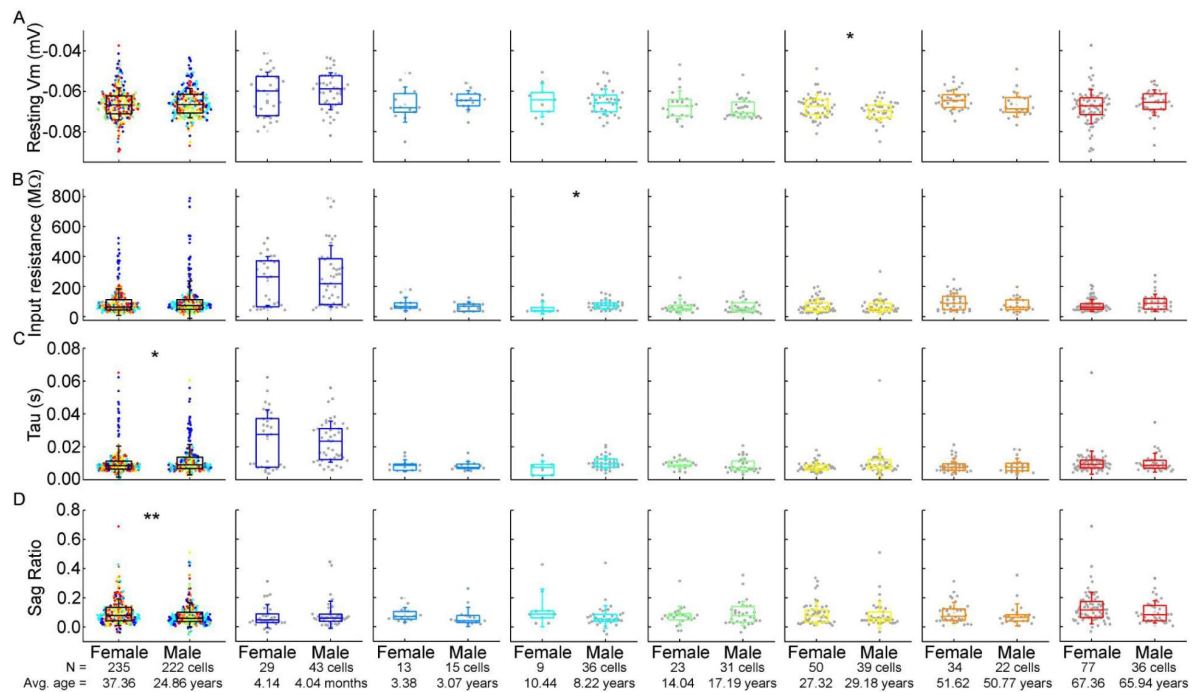
A-C : Box plots showing: F-I slope (A), first AP latency (B), and adaptation of APs (C) from patients with tumor removal (tumor) or VP shunt (shunt) surgical procedures. From left to right: data from all age groups, infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood and late adulthood groups. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$, Mann-Whitney test).



Supplementary Figure 17.

Morphological comparison of the examined cells from patients with tumor removal or VP shunt surgical procedures.

A-H: Boxplots showing morphological features: total (A), apical (B), total basal (C), dendritic length, total number of nodes (D), maximal horizontal (E), and vertical (F) extension, average apical (G) and basal (H) terminal dendritic segment from patients with tumor removal (tumor) or VP shunt (shunt) surgical procedures. From left to right: data from all age groups, infant, early childhood, late childhood, adolescence, middle adulthood and late adulthood groups. Asterisks indicate significance (* $P < 0.05$, Mann-Whitney test).



Supplementary Figure 18.

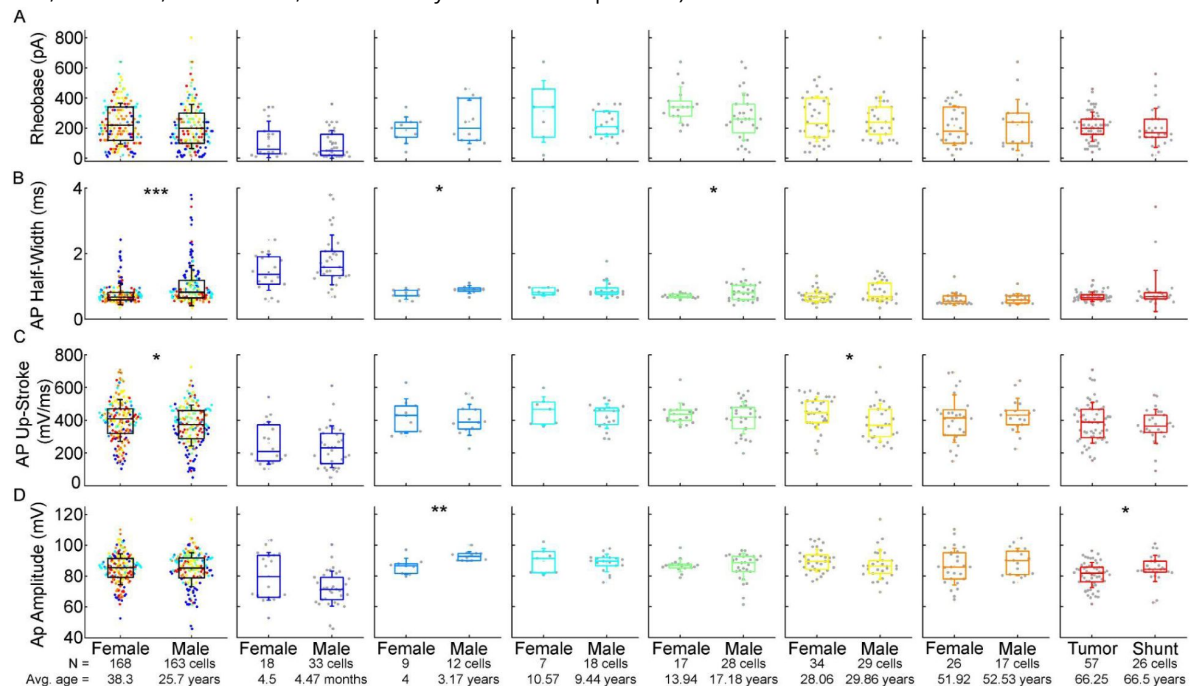
Comparison of subthreshold electrophysiological properties of the examined cells from female and male patients.

A-D : Box plots show resting membrane potential (A), input resistance (B), tau (C), and sag ratio (D) from female and male patients. From left to right: data from all age groups, infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood and late adulthood groups. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$ Mann-Whitney test or two sample t-test).

Supplementary Figure 19.

Comparison of action potential properties of the examined cells from female and male patients.

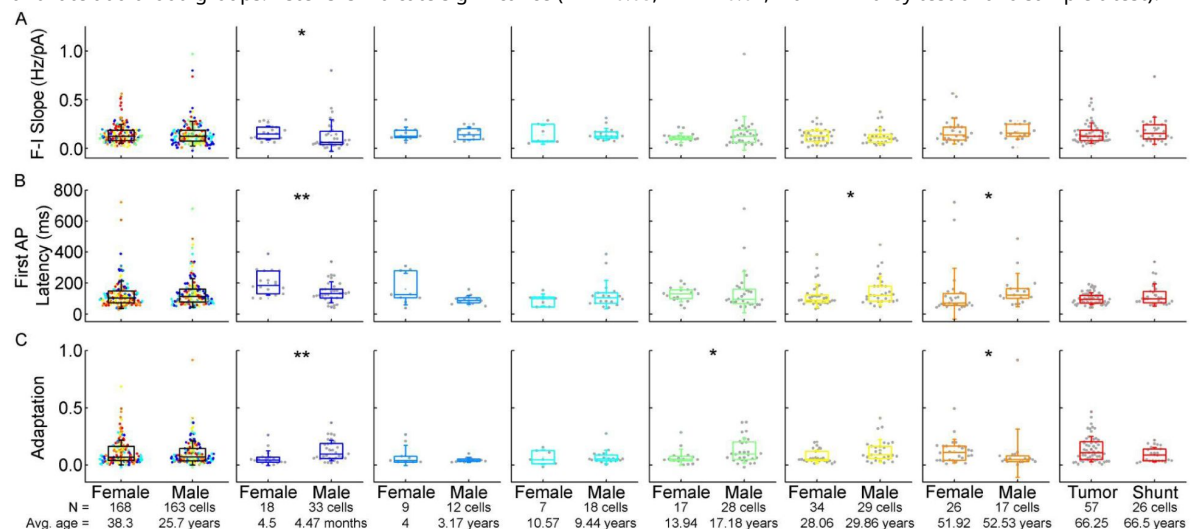
A-D: Boxplots show rheobase (A), action potential half-width mean (B), action potential up-stroke (C) and action potential amplitude (D) from female and male patients. From left to right: data from all age groups, infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood and late adulthood groups. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Mann-Whitney test or two sample t-test).

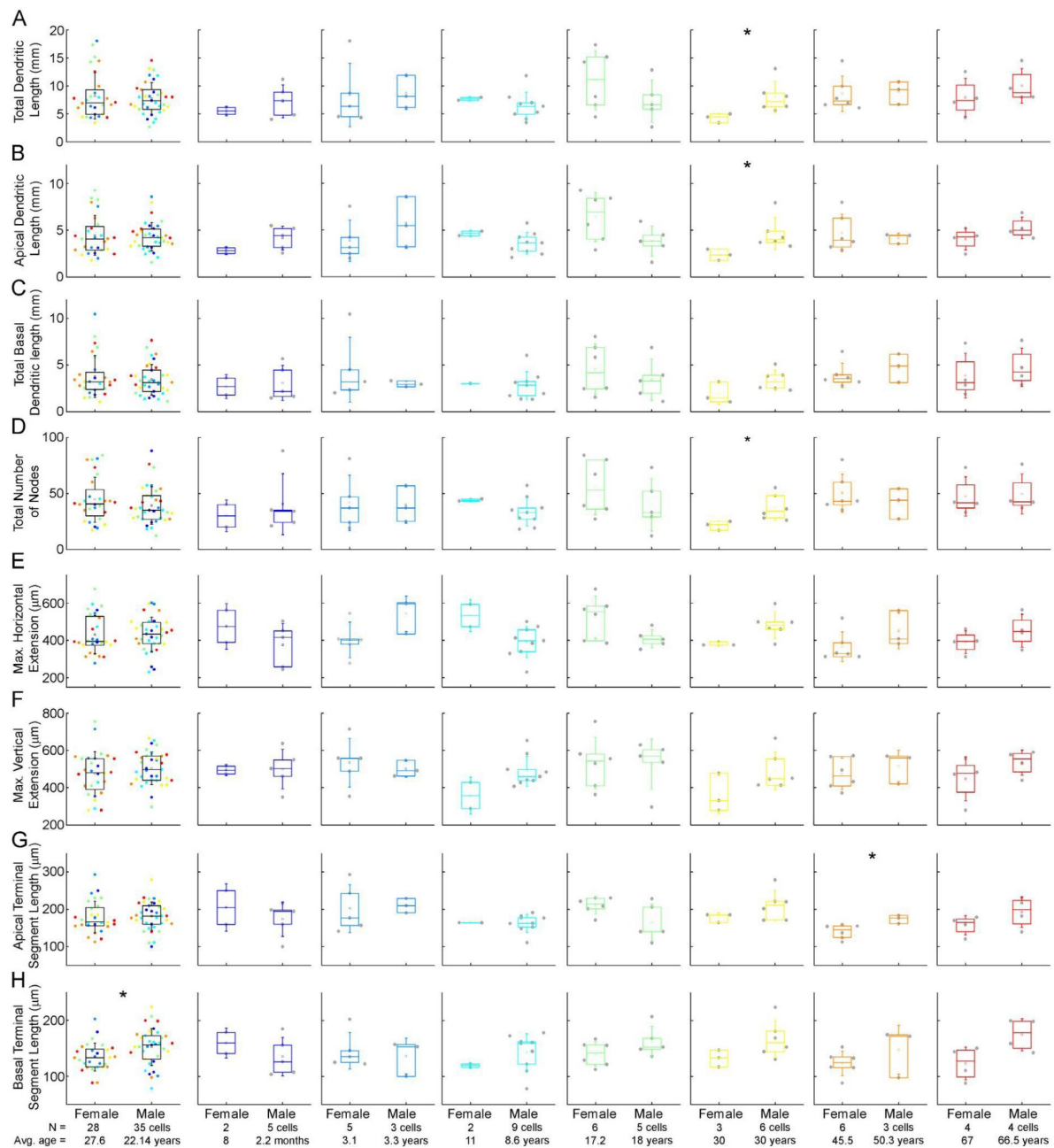


Supplementary Figure 20.

Comparison of firing pattern characteristics of the examined cells from female and male patients.

A-C: Box plots showing: F-I slope (A), first AP latency (B), and adaptation of APs (C) from female and male patients. From left to right: data from all age groups, infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood, and late adulthood groups. Asterisks indicate significance (* $P < 0.05$, ** $P < 0.01$, Mann-Whitney test or two sample t-test).





Supplementary Figure 21.

Morphological comparison of the examined cells from female and male patients.

A-H: Boxplots showing morphological features: total (A), apical (B), total basal (C), dendritic length, total number of nodes (D), maximal horizontal (E), and vertical (F) extension, average apical (G) and basal (H) terminal dendritic segment from female and male patients. From left to right: data from all age groups, infant, early childhood, late childhood, adolescence and late adulthood groups.

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Reviewer #1 (Public review):

Summary:

The manuscript co-authored by Pál Barzó et al is very clear and very well written, demonstrating the electrophysiological and morphological properties of the human cortical layer 2/3 pyramidal cells across a wide age range, from age 1 month to 85 years using whole-cell patch clamp. To my knowledge, this is the first study that look at the cross-age differences biophysical and morphological properties of human cortical pyramidal cells. The community will also appreciate the significant effort involved in recording data from 485 cells, given the challenges associated with collecting data from human tissue. Understanding the electrophysiological properties of individual cells, which are essential for brain function, is crucial for comprehending human cortical circuits. I think this research enhances our knowledge of how biophysical properties change over time in the human cortex. I also think that by building models of human single cells at different ages using these data, we can develop more accurate representations of brain function. This, in turn, provides valuable insights into human cortical circuits and function and helps in predicting changes in biophysical properties in both health and disease.

Strengths:

The strength of this work lies in demonstrating how the electrophysiological and morphological features of human cortical layer 2/3 pyramidal cells change with age, offering crucial insights into brain function throughout life.

Comments on revisions:

Thanks to the authors for addressing my comments and providing greater clarity in the methodology. The analysis is much clearer now. I also appreciate their additional data analysis, particularly on morphology, which strengthens the paper.

<https://doi.org/10.7554/eLife.100390.2.sa3>

Reviewer #2 (Public review):

Summary:

In this study, Barzo and colleagues aim to establish an appraisal for the development of basal electrophysiology of human layer 2/3 pyramidal cells across life and compare their morphological features at the same ages.

Strengths:

The authors have generated recordings from an impressive array of patient samples, allowing them to directly compare the same electrophysiological features as a function of age and other biological features. These data are extremely robust and well organised.

The authors group patient ages into developmentally organised bins, which are elaborated on in supplementary analysis - exemplifying the importance of determining early postnatal development on human neuron function

Weaknesses:

The author's use of (perhaps) arbitrary categorisation of spine morphology could limit the full usefulness of these data.

Overall, the authors achieve their aims by assessing the physiological and morphological properties of human L2/3 pyramidal neurons across life. Their findings have extremely important ramifications for our understanding of human brain development and implications for how different neuronal properties may influence life and disease associated with neurological conditions.

Comments on revisions:

Overall, the authors have satisfied my concerns. I fully appreciate their candour with their data and the potential limitations. I especially appreciate their supplementary data inclusions which I believe truly strengthen their conclusions and are a valuable resource for the field,

I agree whole-heartedly with the authors' assertion that it is perhaps better to use the most sophisticated equipment, not always being most appropriate. However, statistical rigour should still be standard. As such, my one remaining concern relates to inappropriate replicate choice of spine morphology data in figure 6. I commend the authors' inclusion of additional reconstructions and morphology data from further cells in this data set. However, to me, these still represent data from 3 cells and 1 patient/age - as to the best of my interpretation. I feel it would be more helpful to plot cell averages $\pm$ SD for each cell - even if side-by-side with data from all spines. Likewise, it is unclear what statistical test was performed on these data and did it take into account the fact that these values are a) from 3 technical replicates per group, or b) that many of the data sets consist of many zero-values (would a categorical test be more appropriate?).

<https://doi.org/10.7554/eLife.100390.2.sa2>

Reviewer #3 (Public review):**Summary:**

To understand the specificity of age-dependent changes in the human neocortex, this paper investigated the electrophysiological and morphological characteristics of pyramidal cells in a wide age range from infants to the elderly.

The results show that some electrophysiological characteristics change with age, particularly in early childhood. In contrast, the larger morphological structures, such as the spatial extent and branching frequency of dendrites, remained largely stable from infancy to old age. On the other hand, the shape of dendritic spines is considered immature in infancy, i.e., the proportion of mushroom-shaped spines increases with age.

Strengths:

Whole-cell recordings and intracellular staining of pyramidal cells in defined areas of the human neocortex allowed the authors to compare quantitative parameters of electrophysiological and morphological properties between finely divided age groups.

They succeeded in finding symmetrical changes specific to both infants and the elderly, and asymmetrical changes specific to either infants or the elderly. The similarity of pyramidal cell characteristics between areas is unexpected.

Weaknesses:

Human L2/3 pyramidal cells are thought to be heterogeneous, as L2/3 has expanded to a high degree during the evolution from rodents to humans. However, the diversity (subtyping) is not revealed in this paper.

Comments on revisions:

I believe that the current version has been sufficiently revised based on my comments.

<https://doi.org/10.7554/eLife.100390.2.sa1>

Author response:

The following is the authors' response to the original reviews.

Public Reviews:**Reviewer #1 (Public review):****Summary:**

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single cells at different ages using these data, we can develop more accurate representations of brain function. This, in turn, provides valuable insights into human cortical circuits and function and helps in predicting changes in biophysical properties in both health and disease.

Strengths:

The strength of this work lies in demonstrating how the electrophysiological and morphological features of human cortical layer 2/3 pyramidal cells change with age, offering crucial insights into brain function throughout life.

Weaknesses:

One potential weakness of the paper is that the methodology could be clearer, especially in how different cells were used for various electrophysiological measurements and the conditions under which the recordings were made. Clarifying these points would improve the study's rigor and make the results easier to interpret.

Reviewer #2 (Public review):

Summary:

In this study, Barzo and colleagues aim to establish an appraisal for the development of basal electrophysiology of human layer 2/3 pyramidal cells across life and compare their morphological features at the same ages.

Strengths:

The authors have generated recordings from an impressive array of patient samples, allowing them to directly compare the same electrophysiological features as a function of age and other biological features. These data are extremely robust and well organised.

Weaknesses:

The use of spine density and shape characteristics is performed from an extremely limited sample (2 individuals). How reflective these data are of the population is not possible to interpret. Furthermore, these data assume that spines fall into discrete types - which is an increasingly controversial assumption.

Many data are shown according to somewhat arbitrary age ranges. It would have been more informative to plot by absolute age, and then perform more rigorous statistics to test age-dependent effects.

Overall, the authors achieve their aims by assessing the physiological and morphological properties of human L2/3 pyramidal neurons across life. Their findings have extremely important ramifications for our understanding of human life and implications for how different neuronal properties may influence neurological conditions.

Reviewer #3 (Public review):

Summary:

To understand the specificity of age-dependent changes in the human neocortex, this paper investigated the electrophysiological and morphological characteristics of pyramidal cells in a wide age range from infants to the elderly.

The results show that some electrophysiological characteristics change with age, particularly in early childhood. In contrast, the larger morphological structures, such as

the spatial extent and branching frequency of dendrites, remained largely stable from infancy to old age. On the other hand, the shape of dendritic spines is considered immature in infancy, i.e., the proportion of mushroom-shaped spines increases with age.

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Human L2/3 pyramidal cells are thought to be heterogeneous, as L2/3 has expanded to a high degree during the evolution from rodents to humans. However, the diversity (subtyping) is not revealed in this paper.

Recommendations for the authors:

Reviewer #1 (Recommendations for the authors):

The manuscript co-authored by Pál Barzó et al is very clear and very well written, demonstrating the electrophysiological and morphological properties of the human cortical layer 2/3 pyramidal cells across a wide age range, from age 1 month to 85 years using whole-cell patch clamp. To my knowledge, this is the first study that looks at the cross-age differences in morphological and electrophysiological properties of human cortical pyramidal cells. The community will also appreciate the significant effort involved in recording data from 485 cells, given the challenges associated with collecting data from human tissue. understanding the electrophysiological properties of individual cells, which are essential for brain function, is crucial for comprehending human cortical circuits. I think this research enhances our knowledge of how biophysical properties change over time in the human cortex. I also think that by building models of human single cells at different ages using these data, we can develop more accurate representations of brain function. This, in turn, provides valuable insights into human cortical circuits and function and helps in predicting changes in biophysical properties in both health and disease.

We are grateful for the positive evaluation of our work. We also thank the reviewers for their comments and believe that our manuscript has improved significantly with their help. In addition to the reviewer's suggestions for improvement, further cell reconstructions were performed to make the anatomical data more robust (n = 1,2,3,3,4,3,2 additional reconstruction in age groups infant, early childhood, late childhood, adolescence, young adulthood, middle adulthood and late adulthood, respectively; $\Sigma n = 18$). Four additional cells were added to the spine analysis and the statistics associated with each additional dataset were updated.

I have some comments, particularly regarding the methodology and data presentation, to improve the clarity of the paper

(1) I assume the tissue is from the resected area adjacent to the tumor. Could you please clarify this in the Methods section?

Thank you for this comment, it has been clarified in the Methods section with the following sentence: "We used human cortical tissue adjacent to the pathological lesion that had to be

surgically removed from patients (n = 63 female n = 45 male) as part of the treatment for tumors, hydrocephalus, apoplexy, cysts, and arteriovenous malformation.”

(2) Regarding the presentation of data in the Methods section, could you please clarify whether the authors used different cells for measuring the various electrophysiological properties? The number of recorded cells for calculating subthreshold properties (e.g., late adulthood: n = 113) differs from the number the cells used for calculating suprathreshold properties (e.g., late adulthood: n = 83). If this is the case, it may make it difficult to compare the electrophysiological properties. Could you please clarify this?

The different element numbers are indeed due to the fact that different quality criteria were defined for the analysis of fast and slow signals. For the analysis of fast signals (e.g. AP half-width, AP upstroke velocity, AP amplitude), higher quality requirements were established therefore cells with high series resistance ($> 30 \text{ M}\Omega$) were excluded. We have updated and clarified the recording conditions in the text, figures, and methodology section accordingly.

(3) Additionally, they mentioned that their recordings were done at zero holding current and at more than -50 pA. Could you clarify whether the data from these two sets of experiments were combined? If so, please provide an explanation in the methods section.

Basically, we wanted to determine the parameters of the potential changes of the membrane at rest. However, for technical reasons related to the biological amplifier, in some of the experiments a certain continuous holding current may be present during the measurement (3.5% of all experiments). The holding currents were in the range of -50 pA to +60 pA. Within this range, previously checked on mouse neurons we have not found linear correlation between the electrophysiological properties and the holding current. This is reported in the Methods section.

(4) This section needs revision. It is unclear why different series resistances (R_s) or different cells were used to compute various electrophysiological properties." To calculate passive membrane properties (resting membrane potential, input resistance, time constant, and sag) either cells with series resistance (R_s): $22.85 \pm 9.04 \text{ M}\Omega$ (ranging between $-4.55 \text{ M}\Omega$ and $56.76 \text{ M}\Omega$) and 0 pA holding current (n = 154), or cells with holding current $> -50 \text{ pA}$ ($-7.46 \pm 28.56 \text{ pA}$, min: -49.89 pA , max: 59.68 pA) and $R_s < 30 \text{ M}\Omega$ ($18.96 \pm 6.48 \text{ M}\Omega$) (n = 23) were used. For the analysis of high frequency action potential features (AP half-width, AP up-stroke velocity, AP amplitude and rheobase) cells with $R_s < 30 \text{ M}\Omega$ (n = 331 cells with R_s $19.2 \pm 6.6 \text{ M}\Omega$) and holding current $> -50 \text{ pA}$ (n = 308 with 0 pA holding current and R_s : $19.22 \pm 6.59 \text{ M}\Omega$, n = 23 with holding current: $-7.46 \pm 28.56 \text{ pA}$ and R_s : $18.96 \pm 6.48 \text{ M}\Omega$) were used."

To make the chapter clearer, we simplified the cell groups used to analyse the different electrophysical properties and revised the Method section as follows: “For the analysis of the electrophysiological recordings n = 457 recordings with a series resistance (R_s) of $24.93 \pm 11.18 \text{ M}\Omega$ (max: $63.77 \text{ M}\Omega$) were used. For the analysis of fast parameters related to the action potential (AP half-width, AP upstroke velocity, AP amplitude and rheobase), higher quality requirements were set and cells with $R_s > 30 \text{ M}\Omega$ were excluded. This reduced the data set to n = 331 cells with R_s $19.42 \pm 6.2 \text{ M}\Omega$.”

(5) The authors recorded the sag ratio using a -100 pA injected current. Is there a technical reason why they did not inject more than -100 pA?

There is no particular technical reason, we use similar to others this current amplitude for voltage response recordings over the years to record electrophysiological traces.

(6) In the abstract, the authors mentioned that data were recorded from ages 1 month to 85 years. However, in the results, they stated that data were recorded from ages 0 to 85 years. Could you please clarify this discrepancy?

We corrected this discrepancy.

(7) Additionally, the results mention that data were collected from 485 human cortical layer 2/3 (L2/3) pyramidal cells, but subthreshold membrane features such as resting membrane potential, input resistance, time constant (τ), and sag ratio were calculated in 475 cortical pyramidal cells from 99 patients. Could you please clarify these discrepancies? In the discussion "We recorded from $n = 457$ human cortical excitatory pyramidal cells from the supragranular layer from birth to 85 years"

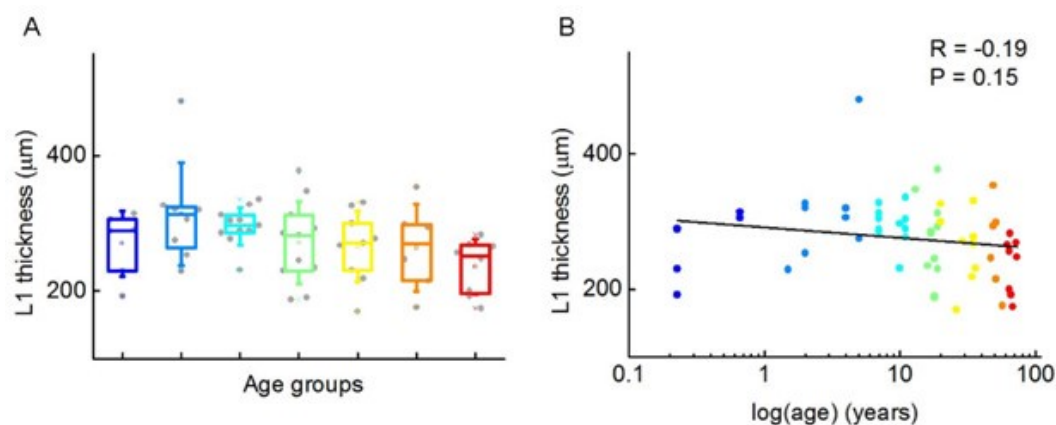
Thank you for pointing this out, we have corrected the error. Although our full data set contained 485 pyramidal cells, 28 recordings were excluded from the electrophysiological analysis and were used for morphological evaluation only, therefore 457 recordings were used for passive parameter measurements.

(8) Regarding the distance from the pia to the border layer L1/L2, did the authors notice any differences across ages?

To investigate whether the thickness of cortical layer 1 changes throughout life, we measured the L1 thickness and found no significant differences between age groups ($P = 0.09$, Kruskal-Wallis test) (Author response image 1).

Author response image 1.

Thickness of cortical layer 1 at different life stages. (A) Boxplot shows the thickness of layer 1. (B) Scatter plot shows the distribution of L1 thickness measured on the reconstructed cells. Age is shown in years on a logarithmic scale, dots are color-coded according to the corresponding age groups.



(9) I am not sure why they referred to the data as layer 2/3 when most of the data, based on Figure 1E, were recorded from a distance of 0-200 μm from the L1/L2 border. Could it be that there is no significant depth-dependent variation in electrophysiological properties, as reported by Berg (2021), Kalmbach (2018), and Chameh (2021)?

Although the vast majority of our data comes from a distance of less than 200 μm from the L1/L2 border, we cannot neglect the fact that our dataset also contains a small number of

cells deeper than this, which are layer 3 cells. Apart from some differences shown in Supplementary Figures 7-9, we found no general difference between cells located at a distance of less than 200 μm and more than 200 μm from the L1 border.

(10) In Figure 1, there is variability in resting membrane potential (RMP), tau, and input resistance (IR) within the infant age group. However, this trend is not observed in the sag ratio. Could you please discuss this finding?

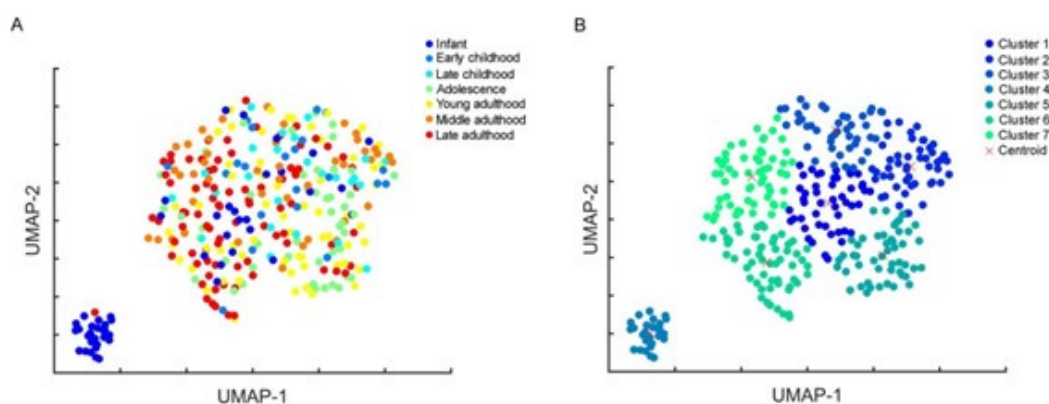
The large variance in the data is due to dramatic changes in these three parameters during the first year of life. Supplementary Figure 3 shows the comparisons of parameter distributions of patients between 0-6 months and 6-12 months. The sag amplitude in these cells is generally low therefore no such large changes could have occurred in them.

(11) Did the authors use a K-Nearest Neighbors (KNN) test to assess the accuracy of the infant cluster in Figure 3F?

Based on eight electrophysiological features of the cells (resting Vm, input resistance, tau, sag ratio, rheobase, AP half-width, AP up-stroke, and AP amplitude), the infant pyramidal cells on a UMAP form a distinct group (Author response image 2A) represented by cluster 4 on Author response image 2B. When calculating the sum of the Euclidean distances of cells within the cluster from the centroid, the isolated infant group (cluster 4) shows the smallest distance value from the centroid (cluster 1: 40.2, cluster 2: 36.21, cluster 3: 39.96, cluster 4: 5.72, cluster 5: 39.2, cluster 6: 55.74, cluster 7: 54.27), demonstrating that infant cells create a discrete cluster distinct from other age groups (Author response image 2B).

Author response image 2.

(A) Uniform Manifold Approximation and Projection (UMAP) of 8 selected electrophysiological properties (resting Vm, input resistance, tau, sag ratio, rheobase, AP half-width, AP up-stroke, and AP amplitude) with data points for 331 cortical L2/3 pyramidal cells, colored with the corresponding age groups. (B) UMAP colored by k-means clustering with 7 clusters, red crosses represent the centroids of the clusters.



(12) Missing citation: 'Previous research has shown that the biophysical properties of human pyramidal cells show depth-related correlations throughout L2/3 (Berg et al., 2021).' Please include citations for Kalmbach (2018) and Chameh (2021).

We thank for the additional references, these studies are now cited.

(13) Have they noticed any morphological properties differences among the different cortical lobes (Parietal, Temporal, Frontal, and Occipital). It would be beneficial to present this data, especially since they have a sufficient sample size from each cortical lobe.

The majority of our data set on the morphological properties of pyramidal cells comes from the parietal (n = 17 cells) and temporal lobe (n = 15). We found no significant differences in the morphological properties of cells from these two brain regions and no differences between age groups in the same cortical lobes.

(14) Have the authors found differences in spine characteristics among different cortical areas, as reported previously by 10.1023/a:1024134312173).

We found morphological differences in dendritic spines in the different brain regions, yet, our data are limited to draw definitive conclusions.

Reviewer #2 (Recommendations for the authors):

Major

(1) I believe that these data presented in all main text figures would be more intuitive to be plotted on a log(age) scale, such as shown in supplementary Figure 13. The bounds of the ages used for different groups, as summarised in Figure 1 feel somewhat arbitrary.

Recent neuroscientific studies on postnatal ageing mainly use the age-group comparison format (Kang 2011, Bethlehem 2022), which has been defined based on milestones in the cognitive, motor, social-emotional, and language/communications domains of observable behaviour (Zubler et al. 2022, for detailed definitions see Kang 2011). Since many parameters do not vary linearly but take a U-shape (or inverted U-shape), statistical quantification of these is not straightforward, so we would retain the age-group format for the main graphs. However, at the reviewer's suggestion, electrophysiological and morphological parameters are presented on a log(age) scale as supplementary figures (Supplementary Figures 2,4 and 6), also further statistical analysis was also carried out without grouping the data (see response 5).

(2) The authors present a lot of data values in the text, which is also shown in the figures. This makes reading of the manuscript somewhat difficult in places. For brevity, it may be best to present this data as supplementary tables.

Thank you for this suggestion. We have inserted these data as tables.

(3) I am unclear why the authors excluded cells that fired doublets or triplets in Figure 4? Were these included in the passive and AP-specific analysis - but excluded from F-I plots? Please clarify the rationale and the relative abundance of these physiological types based on age - one might predict that more initial-burst firing types are associated with older neurons?

Thank you for drawing attention to this anomaly. We have updated the figures and text by adding the cells with initial burst firing. These cells are also included in the analysis of passive and action potential properties. In our overall dataset, 6.78% of cells show burst firing; infant: 0%, early childhood: 3.57% (1 cell), late childhood: 0%, adolescence: 11.11% (6 cells), young adulthood: 10.11% (9), middle adulthood: 10.71% (6 cells), late adulthood: 7.96 (9 cells) of all cells including the age groups.

(4) The statistical analyses performed in Figure 6 are not justified. From the authors' description of these data, they derive spine density measurements from 1 infant and 1 aged adult, then perform pseudoreplicated analysis in these individuals. These data would require greater replication from infant and aged groups - with the possible inclusion of a younger adult group also. It would be ideal to have n=3/age group to allow robust statistical analysis.

Thank you for this point. Accordingly, we have expanded our data set to include n = 3 infant pyramidal cells (83 days old, from one patient) and n = 3 pyramidal cells from three late adulthood patients (64.3 ± 2.08 years old).

(5) Given the high number of individuals and replicates throughout this manuscript, a more circumspect approach to statistics would be appreciated, e.g. a generalised linear mixed effects model - with age as a fixed effect and sex, patient, etc as random effects. This may reveal the greatest statistical power of these important and rich data.

Of the generative models we used the Generalized Additive Mixed Model (GAMM) to describe the relationship between age and the various passive and active electrophysiological features. We defined age with cubic spline smoothing term as the fixed effect and gender, brain area, surgical procedure, and hemisphere as random effects. With GAMM we found that the age-dependent correlation of the examined parameters (resting membrane potential, input resistance, tau, sag ratio, rheobase current, AP half-width, AP up-stroke velocity, AP amplitude, first AP latency, adaptation) was significant, except for F-I slope, described by the model incorporating the four random effects. We also observed correlation with gender, brain area, hemisphere, and surgical procedure in various intrinsic properties. The Author response table 1 below shows the statistical values of GAMM and the statistical tests used in the manuscript to compare.

Author response table 1.

Statistical significance of patient attributes *In the pairwise comparison, the age of cells in the two groups was significantly different: female (subthreshold: 37.36 ± 26.25 years old, suprathreshold: 38.3 ± 25.6 y.o.) - male (subthreshold: 24.86 ± 23.7 y.o., suprathreshold: 25.7 ± 23.93 y.o.), subthreshold: $P = 1.96 \times 10^{-6}$, suprathreshold: $P = 3.25 \times 10^{-5}$ Mann-Whitney test.

**In the pairwise comparison, the age of cells in the two groups was significantly different: surgical procedure: tumor removal (subthreshold: 33.72 ± 24.33 y.o., suprathreshold: 36.43 ± 27.07 y.o.) - VP shunt (subthreshold: 27.38 ± 29.69 y.o., suprathreshold: 27.07 ± 29.37 y.o.) subthreshold: $P = 3.68 \times 10^{-3}$, suprathreshold: $P = 1.64 \times 10^{-3}$, Mann-Whitney test)

Electrophysiological parameters	Age P-value	Age group P-value	Gender P-value	Gender (all age groups)	Brain area P-value	Hemisphere P-value	Surgical procedure P-value	Surgical procedure (all age groups)
Statistical analysis	GAMM	Kruskal - Wallis	GAMM	Pairwise*	GAMM	GAMM	GAMM	Pairwise** (tumor-shunt)
Resting Vm (mV)	1.23×10^{-6}	3.53×10^{-8}	0.95	0.68	5.61×10^{-5}	0.0498	0.025	0.11
Input resistance (MΩ)	$< 2 \times 10^{-16}$	1.29×10^{-16}	0.75	0.2	$< 2 \times 10^{-16}$	4.98×10^{-4}	1.03×10^{-4}	0.02
Tau (ms)	$< 2 \times 10^{-16}$	1.31×10^{-15}	0.44	0.03	4.57×10^{-7}	0.09	3.57×10^{-4}	0.28
Sag ratio	$< 2 \times 10^{-16}$	5.2×10^{-15}	0.44	0.003	0.09	4.7×10^{-3}	3.57×10^{-4}	0.34
Rheobase (pA)	$< 2 \times 10^{-16}$	8.71×10^{-12}	0.68	0.13	0.016	0.4	0.87	9.58×10^{-3}
AP half-width (ms)	$< 2 \times 10^{-16}$	9.57×10^{-25}	6.8×10^{-4}	1.5×10^{-6}	1.03×10^{-5}	0.31	0.61	5.91×10^{-5}
AP up-stroke (mV/ms)	3.21×10^{-3}	1.63×10^{-12}	0.83	0.03	3.21×10^{-3}	0.02	0.012	0.03
AP amplitude (mV)	$< 2 \times 10^{-16}$	2.24×10^{-12}	0.42	0.86	4×10^{-5}	0.11	0.74	0.14
F-I slope	0.055	0.055	0.97	0.6	0.02	0.07	0.7	0.6
First AP latency (ms)	0.01	7.67×10^{-4}	0.59	0.17	0.15	0.89	0.8	0.71
Adaptation	0.03	0.032	0.46	0.95	0.002	0.28	0.34	0.88

(6) Regarding the morphological diversity of dendritic spines. There is some debate in the field as to whether the distinction of specific dendritic spine types - as conveyed in this manuscript - are true subtypes or reflect a continuum of diverse morphology (see Tønneson et al., 2014 Nature Neuroscience). It is appreciated that the approach taken by the authors is the dogma within the field - however, dogma should continue to be challenged. Given that the authors have used DAB labelling combined with light microscopy, the possibility of accurately measuring spine morphology required for determining this continuum is extremely limited (e.g. Li et al., (2023) ACS Chemical Neuroscience). I would suggest that alongside the inclusion of further replicates for their spine analysis, the authors tone down their discussion of spine subtypes given the absence of any synaptic data presented in this current study to support the maturation (or otherwise) of dendritic spine synapses.

Many thanks to the reviewer for this comment. We agree with the drawbacks of our method for testing spine categorization. To increase the reliability of our results, we increased the number of pyramidal cells in the infant and late adult groups. We also revised the figure and as suggested by Reviewer#3 added photos of spines to each category in addition to schematic drawings to give an impression of the phenotype. In the discussion, we only address the differences between two readily separable mushroom and filopodial forms and highlight results that only confirm findings already known in the literature. Although the concerns are valid, we apply the sentence from the above Li et al. (2023) reference “...the most sophisticated equipment may not always be necessary for answering some research questions”. We believe that it is worth sharing our data and the somewhat subjective grouping, which we hope to report in more detail in the future.

Minor

(1) The order of the supplemental materials is out of order with their introduction in the text. These should be revised to reflect the order mentioned in the text.

Thank you for your comment, we have corrected the order of the supplementary figures.

(2) In Supplementary Figure 13, it would be informative to include some form of linear regression to confirm whether an age-dependent effect on neuronal morphology exists.

We have added linear regression to the figure.

(3) Figure 3D = should this be AP - not Ap?

Thank you for drawing attention to this, we have corrected the incorrect typing on the figure.

(4) For UMAP analysis in Figure 3, please provide a table of the features that were used for the 32 & 8-parameter UMAPs respectively.

We have added a table to the Materials and methods section of all the electrophysiological features included in the UMAP.

(5) For morphology, please include pia and L1/2 border for reconstructions shown for clarity.

We indicated both the pia mater and the L1/2 border on the figure showing all the reconstructions (Supplementary Figure 10).

Reviewer #3 (Recommendations for the authors):

Major:

(1) Data were obtained from different cortical areas of human patients of different ages. The electrophysiological characteristics were largely independent of other attributes such as disease, gender, and cortical areas (Supplementary Figure 2). To support the conclusion that age is one of the key attributes responsible for change, a similar morphological analysis would be necessary for gender.

We updated the text and the supplementary section with Supplementary Figures 18-21. to determine if age-related differences in biophysical characteristics are affected by the patient's gender.

(2) 'mushroom-shaped, thin, filopodial, branched, and stubby spines'

Show photographs of individual typical spine types to make the classification easier to understand.

To make the classification more understandable, we have updated the corresponding figure (Figure 6) with representative photos of the dendritic spine types.

(3) Some electrophysiological parameters of the infant group showed higher deviations compared to other age groups. A UMAP (Supplementary Figure 2) shows that some infant neurons form a small cluster, while other infant neurons are scattered with neurons of other ages. Are there any differences between infant neurons in the small cluster and other infant neurons with respect to attributes other than age?

For most of the electrophysiological parameters, the infant age group showed age-dependent variability, as illustrated in Supplementary Figures 3, 2,4 and 6 . The small group of infant cells is not clustered by gender, brain region, or medical condition, as shown in Supplementary Figure 5.

(4) A recent paper (Benavides-Piccione et al. 2024, doi:10.1093/cercor/bhae180) reported that some morphological parameters of human layer 3 neurons differ between occipital and temporal regions. Area-dependent morphological differences have been also reported in non-human primates. Discussion of potential contradictions may therefore be requested.

Most of the cells we reconstructed originated from the parietal and temporal regions (parietal: n = 20, temporal: n = 23, frontal: n = 15, occipital: n = 5). We found no differences in morphological features between these two regions, and we also found no significant differences when we compared the cells from the same brain regions by age group.

(5) L2/3 cells of rodents are morphologically differentiated according to cortical depth. If individual L2/3 cells of humans are less differentiated than those of rodents, this point should be discussed.

Depth-related morphological heterogeneity has already been reported previously (Berg 2021), however, our dataset on the morphological characteristics of pyramidal cells is from the upper L2/3 region, with their soma located at a distance of $117.85 \pm 65.3 \mu\text{m}$ (between: 11.05 and 243.3 μm) from the L1/L2 border. Therefore, we cannot conclude from our data whether humans are less differentiated than rodents.

Minor:

(1) Cell body morphology may affect electrophysiological properties. However, morphological quantification of cell bodies has not been reported. It may be added.

In our DAB-labeled samples, we could not perfectly measure the total volume of the cell body in the reconstructions, therefore our measurements regarding the soma morphology are not shown in the manuscript. When comparing the cell body area of the middle sections of the soma of the reconstructed cells between the age groups, we found no significant differences ($P = 0.082$, Kruskal–Wallis test).

(2) 'The adaptation of the AP frequency response'

Describe how this parameter was obtained.

The adaptation of the AP frequency response or adaptation was calculated as the average adaptation of the interspike interval between consecutive APs.

(3) 'we excluded cells showing initial duplet or triplet action potential bursts'

Why were the burst cells excluded from the analysis?

We have modified the figures and text to include cells with initial burst firing.

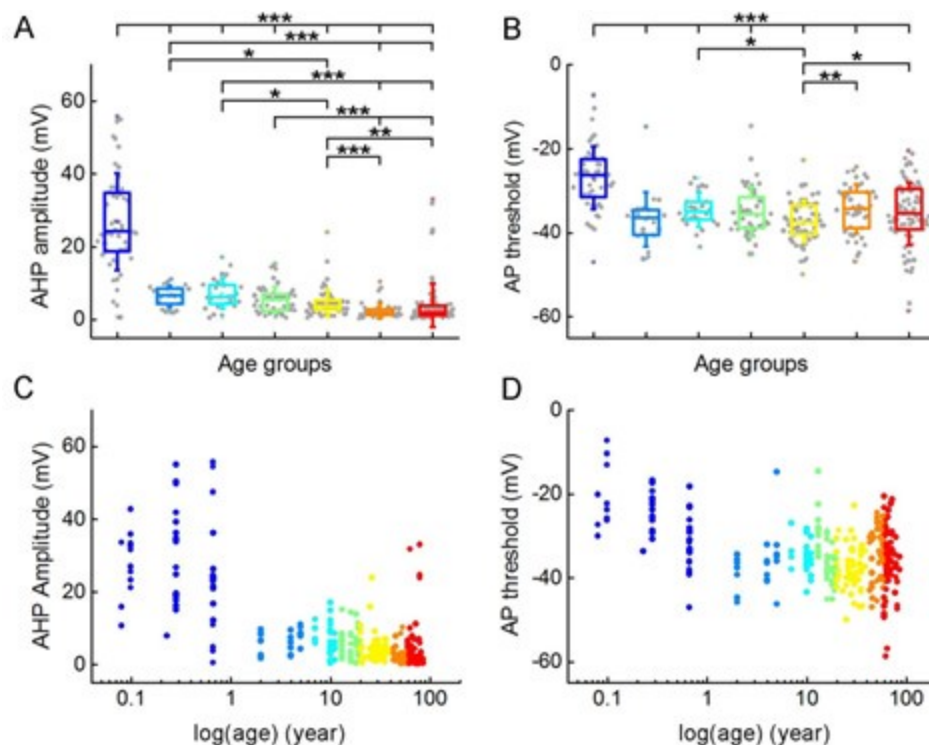
(4) Electrophysiological characteristics to be analyzed:

Spike thresholds and afterhyperpolarizations

We found age-related differences in the amplitude of the afterhyperpolarization ($P = 2.56 \cdot 10^{-30}$, Kruskal–Wallis test) and in the threshold of the action potential ($P = 5.24 \cdot 10^{-12}$, Kruskal–Wallis test) (Author response image 3).

Author response image 3.

Age-dependence of afterhyperpolarization and AP threshold. (A-B) Boxplots show the differences in afterhyperpolarization (AHP) amplitude (A) and AP threshold (B) between age groups. Asterisks indicate statistical significance (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Kruskal-Wallis test with post-hoc Dunn test). (C-D) Scatter plots show AHP amplitude (C) and AP threshold (D) across the lifespan. Age is shown on a logarithmic scale, dots are colored according to the corresponding age group.



(5) 'We identified and labeled each spine on $n = 2$ fully 3D-reconstructed cells'

To which cortical area do these cells belong?

At what depths are they distributed?

Is it possible to report the number of spines, in addition to the density per unit length?

We increased the number of cells in which we analyzed dendritic spine density. The data shown in Figure 6. are from pyramidal cells from an infant patient ($n = 3$ from a single patient) and late adulthood patients ($n = 3$ from 3 patients) (Supplementary Figure 13). The infant cells are from the same patient, the sample is from the right parietal lobe, and the patient is 83 days old. The older cells are from three different patients (#1: 65 years old, right temporal lobe; #2: 66 years old, right parietal lobe; #3: 62 years old, right frontal lobe). Infant cells are located $144.43 \pm 45.26 \mu\text{m}$ (#1: 109.3, #2: 128.49, #3: 195.5 μm), late adult cells $161.22 \pm 66.22 \mu\text{m}$ (#1: 183.5, #2: 213.42, #3: 86.73 μm) from the L1/2 border. We provide the number of spines in an additional supplementary table (Supplementary table 2.).

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