Table 17. Passive membrane properties of LEC III pyramidal neurons, separated into YN, AN, YC, YP, AU, and AI.

Group	RMP (mV)	R_{input} (MΩ)	
YN	−72.28 ± 1.12	54.49 ± 1.39	
AN	−70.73 ± 0.89	60.39 ± 2.91	
Group	**First AP Threshold (mV)**	**Last AP Threshold (mV)**	**p-Value**
YN	-41.54 ± 0.33	-41.63 ± 0.33	AP Threshold, $F_{1,\ 379} = 0.6896$, $p = 0.4068$
			Group, $F_{1,\ 379} = 8.744$, $p = 0.003$**
			Group x AP Threshold, $F_{1,\ 379} = 2.902$, $p = 0.0893$~
AN	-43.40 ± 0.48	-43.15 ± 0.53	YN vs. AN First AP Threshold, $p = 0.0028$**
			Last AP Threshold, $p = 0.0178$*
Group	**First AP Half-Width (ms)**	**Last AP Half-Width (ms)**	**p-Value**
YN	1.97 ± 0.04	2.00 ± 0.03	AP Half-Width $F_{1,\ 379} = 8.761$, $p = 0.0033$**
			Group, $F_{1,\ 379} = 2.667$, $p = 0.1033$
			Group x AP Half-Width $F_{1,\ 379} = 3.120$, $p = 0.0781$~
AN	1.83 ± 0.04	1.95 ± 0.04	YN vs AN First AP Half-Width, $p = 0.0523$~
			Last AP Half-Width, $p = 0.7060$
Group	**First AP Amplitude (mV)**	**Last AP Amplitude (mV)**	**p-Value**
YN	83.61 ± 0.44	80.45 ± 0.43	Two-Way ANOVA, AP Amplitude $F_{1,\ 379} = 521.7$, $p < 0.0001$***
			Group, $F_{1,\ 379} = 0.001639$, $p = 0.9677$
			Group x AP Amplitude $F_{1,\ 379} = 0.3927$, $p = 0.5313$
AN	83.49 ± 0.64	80.50 ± 0.73	YN vs AN First AP Amplitude, $p = 0.9862$
			Last AP Amplitude, $p = 0.9970$
Group	**First AP dV/dt max (v/s)**	**Last AP dV/dt max (v/s)**	**p-Value**
YN	199.3 ± 3.0	175.4 ± 2.9	dV/dt Max $F_{1,\ 379} = 385.7$, $p < 0.0001$***
			Group, $F_{1,\ 379} = 0.4711$, $p = 0.4929$
			Group x dV/dt Max $F_{1,\ 379} = 0.1451$, $p = 0.7034$
AN	202.3 ± 4.2	179.3 ± 4.6	YN vs AN First dV/dt Max, $p = 0.8083$
			Last dV/dt Max, $p = 0.6975$
Group	**RMP (mV)**	**R_{input} (MΩ)**	
YC	-75.35 ± 0.71	55.74 ± 2.53	
YP	-74.37 ± 0.86	57.66 ± 3.70	
AU	-72.71 ± 0.76	59.87 ± 2.27	
AI	-70.24 ± 1.40	59.06 ± 2.95	
Group	**First AP Threshold (mV)**	**Last AP Threshold (mV)**	**p-Value**
YC	-37.33 ± 0.40	-37.39 ± 0.47	AP Threshold, $F_{1,\ 643} = 15.71$, $p < 0.0001$***

Table 17 continued on next page

Table 17 continued

Group	RMP (mV)	R_{input} (MΩ)			
YP	-40.70 ± 0.36	-41.46 ± 0.35	Group, $F_{3,\ 643}$ = 20.74, p < 0.0001***		
			Group x AP Threshold, $F_{3,\ 643}$ = 1.826, p = 0.1412		
AU	-40.80 ± 0.35	-41.10 ± 0.34	Comparison	First AP	Last AP
			YC vs YP	p < 0.0001***	p < 0.0001***
			YC vs AU	p < 0.0001***	p < 0.0001***
			YC vs AI	p < 0.0001***	p < 0.0001***
AI	-40.69 ± 0.39	-41.17 ± 0.32	YP vs AU	p = 0.9974	p = 0.9062
			YP vs AI	p > 0.9999	p = 0.9488
			AU vs AI	p = 0.9947	p = 0.9990
Group	**First AP Half-Width (ms)**	**Last AP Half-Width (ms)**	**p-Value**		
YC	2.23 ± 0.06	2.32 ± 0.09	AP Half-Width, $F_{1,\ 643}$ = 4.928e-005, p = 0.9944		
YP	1.96 ± 0.05	1.94 ± 0.04	Group, $F_{3,\ 643}$ = 28.46, p < 0.0001***		
			Group x Half-Width $F_{3,\ 643}$ = 1.650, p = 0.1767		
AU	1.82 ± 0.04	1.80 ± 0.04	Comparison	First AP	Last AP
			YC vs YP	p = 0.0007**	p < 0.0001***
			YC vs AU	p < 0.0001***	p < 0.0001***
			YC vs AI	p < 0.0001***	p < 0.0001***
			YP vs AU	p = 0.1239	p = 0.1198
AI	1.82 ± 0.04	1.77 ± 0.03	YP vs AI	p = 0.1361	p = 0.0494*
			AU vs AI	p > 0.9999	p = 0.9682
Group	**First AP Amplitude (mV)**	**Last AP Amplitude (mV)**	**p-Value**		
YC	80.02 ± 1.11	76.10 ± 1.07	AP Amplitude, $F_{1,\ 643}$ = 286.5, p < 0.0001		
YP	81.80 ± 0.88	78.92 ± 0.82	Group, $F_{3,\ 643}$ = 3.996, p = 0.0078		
			Group x AP Amplitude, $F_{3,\ 643}$ = 5.083, p = 0.0017		
AU	83.10 ± 0.66	80.27 ± 0.52	Comparisons	First AP	Last AP
			YC vs YP	p = 0.5087	p = 0.1274
			YC vs AU	p = 0.0404*	p = 0.0019**
			YC vs AI	p = 0.9990	p = 0.2916
AI	80.18 ± 0.83	78.19 ± 0.75	YP vs AU	p = 0.6715	p = 0.6391
			YP vs AI	p = 0.5082	p = 0.9233
			AU vs AI	p = 0.0237*	p = 0.1762
Group	**First AP dV/dt max (v/s)**	**Last AP dV/dt max (v/s)**	**p-Value**		
YC	178.0 ± 5.1	151.1 ± 4.6	AP dV/dt max, $F_{1,\ 643}$ = 15.71, p < 0.0001		
YP	183.5 ± 5.3	163.8 ± 4.6	Group, $F_{3,\ 643}$ = 20.74, p < 0.0001		
			Group x AP dV/dt max, $F_{3,\ 643}$ = 1.826, p = 0.1412		
AU	191.0 ± 3.7	172.4 ± 2.9	Comparison	First AP	Last AP
			YC vs YP	p = 0.8306	p = 0.2052

Table 17 continued on next page

Table 17 continued

Group	RMP (mV)	R_{input} (MΩ)			
AI	176.7 ± 4.2	162.0 ± 3.2	YC vs AU	p = 0.1219	p = 0.0017**
			YC vs AI	p = 0.9966	p = 0.2653
			YP vs AU	p = 0.5757	p = 0.4517
			YP vs AI	p = 0.6563	p = 0.9888
			AU vs AI	p = 0.0313*	p = 0.1836

All statistical analyses were first performed with a Two-Way ANOVA. *Post hoc* multiple comparisons tests performed on behaviorally naïve Young and Aged data were done with Sidak's tests. Multiple comparisons performed on YC, YP, AU, and AI data were done with Tukey's tests.

MΩ that remained stable throughout the recording, and an action potential height of at least 70 mV above the holding potential. R_{input} was calculated by a linear fit of the steady state voltage response (average membrane potential 700–900 ms of the 1 s current step) vs current steps (−0.3 to 0 nA, 0.05 nA increments). Action potential properties (i.e. threshold, half-width, amplitude, dV/dt max) were measured from the first action potential evoked by persistent firing and the last action potential recorded in the sweep (***Table 17***). Recordings were acquired and analyzed using pClamp v10 and digitized using a Digidata 1440A analog-to-digital converter. Custom routines using Python were written to analyze R_{input}, and action potential threshold, half-width, amplitude, and dV/dt.

Persistent firing recordings

All persistent firing recordings were performed using patch electrodes containing (in mM): 120 K-Gluconate, 10 HEPES, 0.2 EGTA, 20 KCl, 2 $MgCl_2$, 7 PhCreat di(tris), 4 Na-ATP, 0.3 GTP, and 0.1% biocytin (pH adjusted to 7.3 with KOH, 280 ± 5 mOsm), as described in ***Yoshida et al., 2008***; ***Yoshida and Hasselmo, 2009***. Intrinsic recordings of excitability (i.e. postburst AHP) were performed using patch electrodes (5–8 MΩ) containing (in mM): 120 $KMeSO_4$, 10 KCl, 10 Hepes, 4 Mg_2ATP, 0.4 NaGTP, 10 Na_2phosphocreatine, 0.5% neurobiotin, pH adjusted to 7.3 with KOH, 280 ± 5 mOsm. The experimenters were not able to elicit persistent firing using solutions containing 120 $KMeSO_4$.

Persistent firing was evoked by bath applying 10 µM carbachol (***Tahvildari et al., 2007***; ***Tahvildari et al., 2008***; ***Yoshida et al., 2008***; ***Yoshida and Hasselmo, 2009***). Persistent firing was evoked with three different types of protocols: 1) with a 2 s long training stimulus of 100 pA, 150 pA, and 200 pA amplitudes while the cell's membrane potential was held at 2 mV below spontaneous firing threshold and 2) with a 2 s long train of 2 ms, 2 nA current injection pulses at 20 Hz while the cell was held at 2 mv below spontaneous firing threshold and 3) with a 250 ms long train of 2 ms, 2 nA current injection pulses at 20 Hz while the cell's membrane potential was held at 2 mV and 5 mV more hyperpolarized than its spontaneous firing threshold. Each cell's unique spontaneous firing threshold was determined before the protocols were evoked and measured at intervals throughout the recording session to ensure threshold had not drifted. Persistent firing activity was measured during 20 s long sweeps. Each protocol was evoked three times per cell and the recorded activity was averaged across the three sweeps. There was an average of 1 min interval between each sweep. An incidence of persistent firing was considered if the firing activity occurred within 10 s after the end of the current injection. The average group probability of persistent firing for each protocol was determined as the averaged probability of all cells, based on the proportion of sweeps each cell persistently fired.

Measurements of the afterdepolarization (ADP) and plateau potential (PP) were made while the cell's membrane potential was held at 10 mV more hyperpolarized than its spontaneous firing threshold to ensure that persistent firing would not be evoked. The ADP and PP was then evoked with a 250 ms long train of 2 ms, 2 nA current injection pulses at 20 Hz. Firing rate analyses were performed only from neurons that were able to persistently fire at least once out of the three sweeps (i.e. if a neuron was at 0% firing ability, that neuron was not included in the frequency analysis.) Firing rate was measured in 1 s bins after the offset of the training stimulus. In bins in which there were no action potentials, a value of 0 was assigned to the bin. The mean firing rate of the neuron was determined by averaging the firing rate across the three sweeps. If the neuron was able to persistently