

An Integrative Framework for Sex-Specific Microglial Morphometry and Phenotyping in Alzheimer's Disease

— Supplementary Information —

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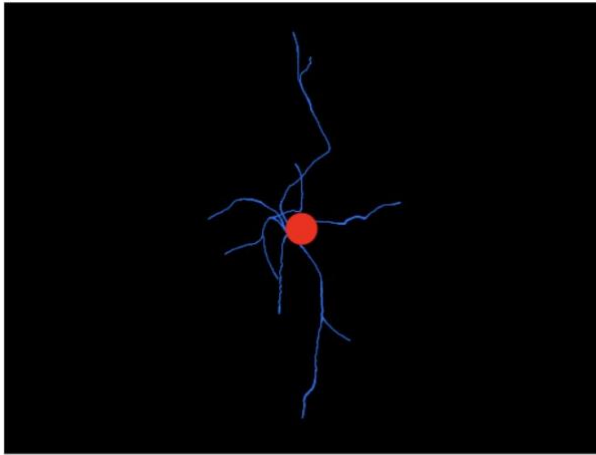
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Column index	Field	Description
1	Index	Sample identifier. A sequential positive integer.
2	Type	Type identifier. A positive integer.
3	X	X-position in micrometers
4	Y	Y-position in micrometers
5	Z	Z-position in micrometers
6	R	Radius in micrometers (half the node thickness)
7	Parent	Parent sample identifier.

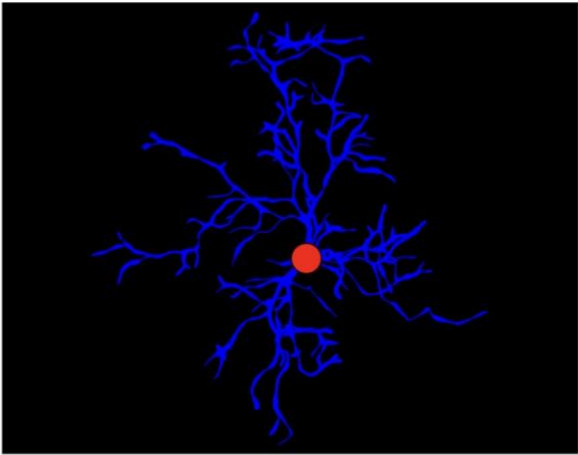
Online Resource 1 SWC column definitions, where each row encodes a single morphological node. Reproduced from the SWC specification documentation (SWC Specification Governing Board 2023), licensed under CC BY-ND 4.0.

Index	Type	X	Y	Z	R	Parent
1	1	0	0	0	1.634	-1
2	1	1.18	0.81	-0.76	1.634	1
3	1	-1.17	-0.81	0.75	1.634	1
4	7	-0.31	0.06	0.3	1.96	1
5	7	-0.56	0.02	0.66	1.961	4
6	7	-0.7	-0.15	0.95	1.757	5
7	7	-0.75	-0.48	1.09	1.536	6

Online Resource 2 Sample SWC data from a representative hippocampal microglia reconstruction (Type: 1 = soma, 7 = glial process; Parent = -1 denotes root node)



(a)



(b)

Online Resource 3 Representative hippocampal microglial reconstructions from NeuroMorpho.org

- (a) Representative reconstruction of a 5xFAD microglia, showing reduced branching complexity relative to control.
- (b) Representative reconstruction of a wild-type control microglia, showing greater morphological elaboration and branching density. Red sphere indicates soma location in both reconstructions.

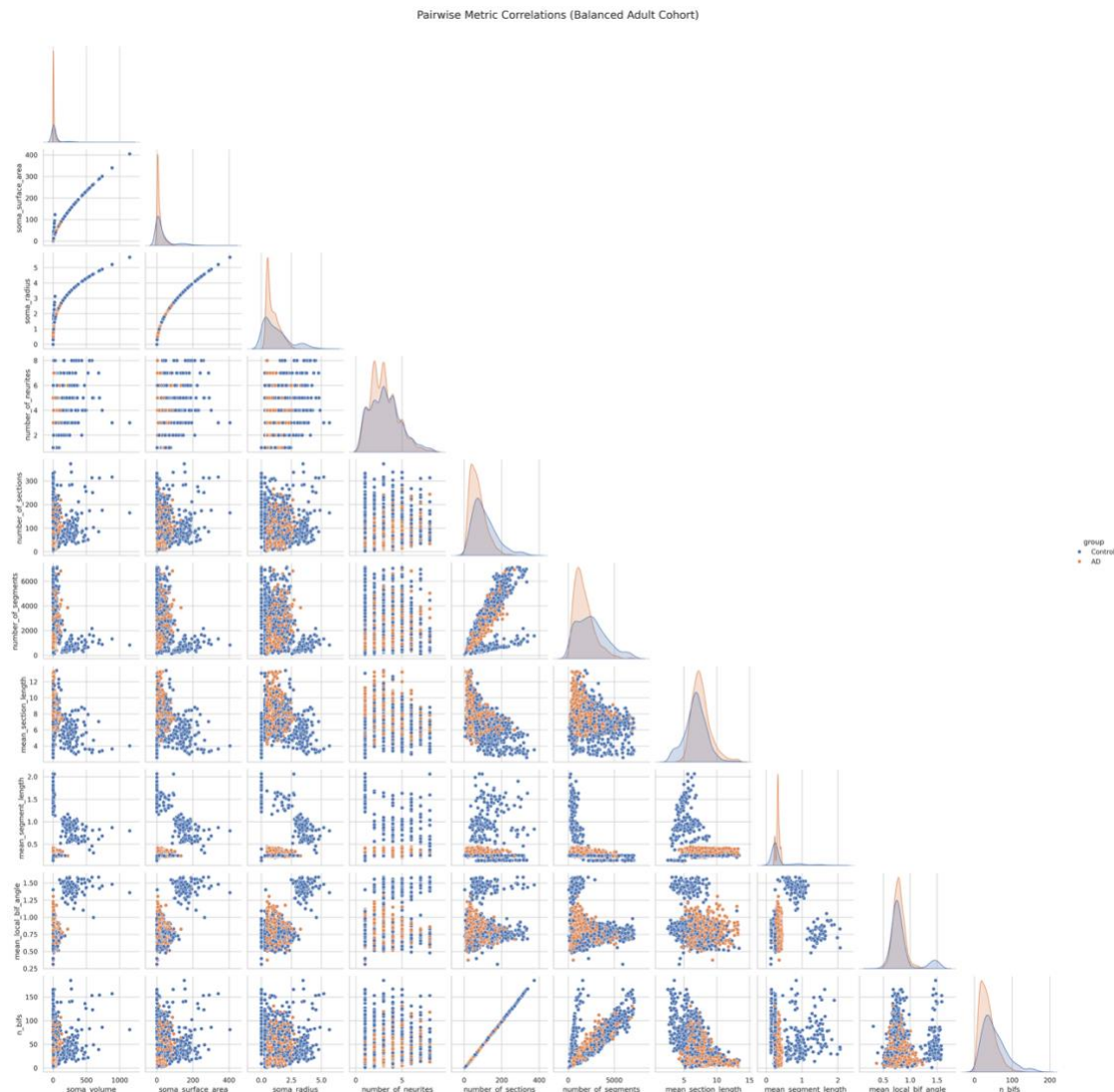
Feature	Description
Soma volume	Total three-dimensional volume of the cell body in cubic micrometers
Soma surface area	Total surface area of the cell body in square micrometers
Soma radius	Mean radius of the cell body in micrometers, derived from the SWC node coordinates
Number of neurites	Total count of primary processes extending directly from the soma
Number of sections	Total count of unbranched cable segments between consecutive branch points or endpoints
Number of segments	Total count of individual SWC edges connecting adjacent nodes across the entire reconstruction
Mean section length	Average length of unbranched sections in micrometers
Mean segment length	Average length of individual node-to-node edges in micrometers
Number of bifurcations	Total count of branch points where a single process divides into two daughter branches
Mean local bifurcation angle	Average angle in degrees between daughter branches at each bifurcation point

Online Resource 4 Morphometric features extracted from microglial SWC reconstructions using the NeuroM Python library (v4.0.4; Blue Brain Project 2024). Features capture soma morphology and branching complexity, which are established indicators of microglial functional state (Kozłowski and Weimer 2012; Reddaway et al. 2023).

Feature	Description
Average total perseverative correct responses	Mean number of continued responses at the correct aperture following successful signal detection and food presentation, reflecting perseverative behavior and inhibitory control deficits
Average accuracy at threshold	Mean percentage of correct aperture responses at the animal's stimulus detection threshold, reflecting sustained attentional capacity under progressively challenging signal conditions
Average task condition at threshold	Mean difficulty level reached at performance threshold, incorporating variations in stimulus duration, location, and pre-cue delay timing, reflecting overall attentional capability
Average trial accuracy	Mean percentage of trials in which the animal correctly detected and responded to the stimulus light within the allotted time window, reflecting overall sustained attention
Average latency for correct responses	Mean time in seconds between stimulus onset and a correct nose-poke in the signaled aperture, reflecting attentional engagement and response speed
Average latency for incorrect responses	Mean time in seconds between stimulus onset and an incorrect nose-poke in a non-signaled aperture, reflecting errors of commission and inattentive responding
Average omission rate	Mean percentage of trials in which the animal failed to respond to the stimulus within the prescribed time limit, reflecting errors of omission and lapses in sustained attention
Average number of premature responses	Mean number of nose-poke responses made before stimulus onset, reflecting motor impulsivity and failure of inhibitory control
Average latency to collect reward	Mean time in seconds between a correct response and retrieval of food from the reward cachet, reflecting motivation and reward processing speed

Online Resource 5 Behavioral metrics extracted from the Five-Choice Serial Reaction Time Task (5CSRTT) via MouseBytes (Beraldo et al. 2019). Metrics capture distinct cognitive domains including sustained attention, motor impulsivity, inhibitory control, and reward motivation, enabling

multidimensional assessment of sex-specific cognitive profiles in 5xFAD and wild-type control mice (Robbins 2002).



Online Resource 6 Pairwise scatter matrix showing correlations among ten morphometric features in the filtered, balanced dataset ($n = 2,210$ cells).

Outside of expected geometric constraints (such as the non-linear, monotonic relationships between soma volume, radius, and surface area), exceptionally tight linear dependencies were observed among branch complexity metrics. Specifically, strong positive correlations were found between the number of sections vs. number of segments, and number of segments vs. number of bifurcations. These relationships manifest as tight, narrow, linear clusters along the scatter diagonals, demonstrating that fundamental branching architectural rules remain tightly coupled across both groups. However, group-specific structural divergence is clearly visible within the diagonal kernel density estimate (KDE) plots. The KDE distributions reveal a systematic, leftward shift in peak density for the 5xFAD AD cohort across branch length, section counts, and bifurcation frequencies. This structural compression confirms that while the mathematical coupling between branch segment and node production is preserved, the 5xFAD disease state is characterized by an overall reduction in total microglial branching capacity and spatial complexity.

Metric	Sex	Control Mean $\pm$ SD	AD Mean $\pm$ SD	% Change from Control	t-stat	p-value	Adjusted p-value (Bonferroni)	Cohen's d
Soma Volume	Male	48.7308 $\pm$ 119.7934	14.8466 $\pm$ 20.1209	-69.53	-8.9812	1.0283E-18	2.0566E-17	-0.3421
Soma Surface Area	Male	33.3407 $\pm$ 56.2858	18.6993 $\pm$ 17.5512	-43.91	-7.7533	1.6981E-14	3.3963E-13	-0.3096
Soma Radius	Male	1.2317 $\pm$ 1.0663	1.0925 $\pm$ 0.5432	-11.31	-3.4414	5.9392E-04	0.011878	-0.1499
Number of Neurites	Male	3.2821 $\pm$ 1.5527	3.3527 $\pm$ 1.4772	2.15	0.8766	0.380885	1.000000	0.0462
Number of Sections	Male	102.0356 $\pm$ 66.0643	82.0388 $\pm$ 43.1165	-19.6	-7.2095	9.0124E-13	1.8025E-11	-0.3357
Number of Segments	Male	2653.7648 $\pm$ 1591.2576	1943.8934 $\pm$ 1060.6390	-26.75	-10.5201	5.5655E-25	1.1131E-23	-0.493
Mean Section Length	Male	7.1699 $\pm$ 1.5454	7.1726 $\pm$ 1.3242	0.04	0.036	0.971321	1.000000	0.0018
Mean Segment Length	Male	0.2811 $\pm$ 0.1256	0.3053 $\pm$ 0.0466	8.63	5.562	3.1504E-08	6.3009E-07	0.2276
Mean Local Bifurcation Angle	Male	0.8280 $\pm$ 0.2477	0.7817 $\pm$ 0.0968	-5.59	-5.3162	1.2164E-07	2.4328E-06	-0.2194
Number of Bifurcations	Male	49.3768 $\pm$ 32.9322	39.3430 $\pm$ 21.4169	-20.32	-7.269	5.8976E-13	1.1795E-11	-0.3381
Soma Volume	Female	44.7684 $\pm$ 99.6562	8.4636 $\pm$ 17.8939	-81.09	-12.9558	1.726E-36	3.4519E-35	-0.4403
Soma Surface Area	Female	33.5200 $\pm$ 53.5397	11.8271 $\pm$ 15.0908	-64.72	-13.7927	3.8881E-41	7.7762E-40	-0.4843
Soma Radius	Female	1.1970 $\pm$ 1.1115	0.8460 $\pm$ 0.4752	-29.33	-9.8835	1.6181E-22	3.2361E-21	-0.3688
Number of Neurites	Female	3.1927 $\pm$ 1.7044	2.7856 $\pm$ 1.3589	-12.75	-5.7685	9.6052E-09	1.921E-07	-0.2545
Number of Sections	Female	116.2854 $\pm$ 66.3596	64.1730 $\pm$ 39.0206	-44.81	-22.0293	4.1242E-96	8.2484E-95	-0.8854
Number of Segments	Female	2516.0734 $\pm$ 1719.5428	1562.3599 $\pm$ 1069.5747	-37.9	-15.1867	3.1857E-49	6.3714E-48	-0.62
Mean Section Length	Female	6.4206 $\pm$ 1.7863	7.9912 $\pm$ 1.4566	24.46	20.9599	4.4414E-86	8.8828E-85	0.9316
Mean Segment Length	Female	0.4439 $\pm$ 0.4227	0.3344 $\pm$ 0.0465	-24.65	-9.3915	2.2834E-20	4.5668E-19	-0.3144
Mean Local Bifurcation Angle	Female	0.8486 $\pm$ 0.2471	0.7941 $\pm$ 0.1130	-6.43	-6.7782	1.5971E-11	3.1942E-10	-0.2564
Number of Bifurcations	Female	56.5463 $\pm$ 33.2597	30.6937 $\pm$ 19.3621	-45.72	-21.8954	4.0002E-95	8.0003E-94	-0.8776

Online Resource 7 Quantitative comparison of male and female morphometric data. Mean $\pm$ SD, percent change from control, effect sizes (Cohen's d), t statistic, and p values from Welch's t-tests (including the adjusted p-value after implementing the Bonferroni correction) are reported for ten morphometric features across AD and control groups.

Metric	Sex	Control Mean $\pm$ SD	AD Mean $\pm$ SD	% Change from Control	t-stat	p-value	Adjusted p-value (Bonferroni)	Cohen's d
Average Trial Accuracy	Male	84.75 $\pm$ 10.28	81.91 $\pm$ 10.43	-3.35	-5.3168	1.2527E-07	2.2549E-06	-0.2745
Average Trial Accuracy	Female	84.73 $\pm$ 9.86	81.28 $\pm$ 11.15	-4.08	-5.5252	4.932E-08	8.8776E-07	-0.3394
Average Omission Rate	Male	20.21 $\pm$ 13.48	22.43 $\pm$ 13.62	11.03	3.1945	0.001436	0.025852	0.1647
Average Omission Rate	Female	19.97 $\pm$ 12.85	20.60 $\pm$ 14.98	3.15	0.7525	0.452054	1.000000	0.047
Average Latency For Correct Responses	Male	1.23 $\pm$ 0.32	1.29 $\pm$ 0.31	4.96	3.784	1.6145E-04	0.002906	0.1927
Average Latency For Correct Responses	Female	1.26 $\pm$ 0.32	1.23 $\pm$ 0.33	-2.56	-1.723	0.085379	1.000000	-0.1004
Average Latency For Incorrect Responses	Male	2.07 $\pm$ 0.84	1.88 $\pm$ 0.77	-9.6	-4.8639	1.2877E-06	2.3179E-05	-0.2446
Average Latency For Incorrect Responses	Female	2.04 $\pm$ 0.85	1.74 $\pm$ 0.83	-14.76	-6.2635	6.7592E-10	1.2167E-08	-0.3558
Average Total Perseverative Correct Responses	Male	9.41 $\pm$ 6.96	6.43 $\pm$ 5.08	-31.65	-9.9197	1.5879E-22	2.8583E-21	-0.4706
Average Total Perseverative Correct Responses	Female	9.08 $\pm$ 6.64	6.62 $\pm$ 4.67	-27.02	-8.1898	8.6845E-16	1.5632E-14	-0.3931
Average Accuracy At Threshold	Male	85.43 $\pm$ 8.72	81.78 $\pm$ 10.09	-4.27	-7.3791	3.1218E-13	5.6193E-12	-0.3944
Average Accuracy At Threshold	Female	85.49 $\pm$ 8.72	81.28 $\pm$ 10.08	-4.93	-7.4892	2.5742E-13	4.6336E-12	-0.4656
Average Task Condition At Threshold	Male	1987.25 $\pm$ 776.72	2107.66 $\pm$ 827.50	6.06	2.8932	0.003883	0.069897	0.1513
Average Task Condition At Threshold	Female	1774.42 $\pm$ 659.34	1902.77 $\pm$ 760.36	7.23	3.0213	0.002627	0.047277	0.1876
Average Number Of Premature Responses	Male	1.19 $\pm$ 0.28	1.21 $\pm$ 0.27	2.15	1.8356	0.066653	1.000000	0.0936
Average Number Of Premature Responses	Female	1.22 $\pm$ 0.27	1.28 $\pm$ 0.32	5.49	3.7731	1.777E-04	0.003199	0.2345
Average Latency To Collect Reward	Male	1.34 $\pm$ 0.67	1.63 $\pm$ 0.95	21.53	6.563	8.6548E-11	1.5579E-09	0.3684
Average Latency To Collect Reward	Female	1.29 $\pm$ 0.71	1.30 $\pm$ 0.69	1.38	0.448	0.654276	1.000000	0.0254

Online Resource 8 Quantitative comparison of male and female 5CSRTT behavioral data. Mean $\pm$ SD, percent change from control, effect sizes (Cohen's d), t statistic, and p values from Welch's t-tests (including the adjusted p-value after implementing the Bonferroni correction) are reported for nine behavioral metrics across AD and control groups.