

Supplemental Table 3.1.

Statistical values for analyses performed on behavioral data. All behavioral datasets were analyzed with either Two-Way ANOVA or Mixed Effects Model with Bonferroni's *post hoc*. Body weight datasets were analyzed using Pearson's R correlations. Datasets from Figure 1.

Dataset		Main Effect		Interactions		Multiple Comparisons		
		F (DFn, DFd)	p-value	F (DFn, DFd)	p-value	Effect	Groups	p-value
Mechanical Allodynia	Males	Time: F (3.816, 82.25) = 18.08	p<0.0001	F (18, 388) = 1.822	p=0.0213	Age	Male Female	*
		Age: F (1, 22) = 7.580	p=0.0116					
	Females	Time: F (4.935, 103.6) = 18.76	p<0.0001	F (18, 378) = 0.9933	p=0.4665			
	Age: F (1, 21) = 1.585	p=0.2219						
	Effect Size	Sex: F (1, 43) = 0.1242	p=0.7263	F (1, 43) = 2.569	p=0.1163			p=0.0044
		Age: F (1, 43) = 8.566	p=0.0055					p=0.7237
Thermal Hyperalgesia	Males	Time: F (3.872, 80.03) = 13.95	p<0.0001	F (6, 124) = 1.606	p=0.1509	Sex	Young Aged	p=0.1184 p=0.0026
		Age: F (1, 22) = 0.9512	p=0.3400					
	Females	Time: F (4.253, 81.52) = 16.22	p<0.0001	F (6, 115) = 1.717	p=0.1231			
	Age: F (1, 21) = 0.7902	p=0.3841						
	Effect Size	Sex: F (1, 43) = 13.59	p=0.0006	F (1, 43) = 0.4797	p=0.4923			
		Age: F (1, 43) = 0.5173	p=0.4759					
Hyperalgesic Priming	Males	Time: F (3.113, 31.90) = 7.153	p=0.0007	F (4, 41) = 0.2845	p=0.8863	Sex	Males Female	p=0.3613 p=0.212
		Age: F (1, 11) = 1.632	p=0.2277					
	Females	Time: F (3.105, 45.98) = 16.36	p<0.0001	F (4, 61) = 1.545	p=0.2006			
	Age: F (1, 16) = 3.525	p=0.0788						
	Effect Size	Sex: F (1, 27) = 2.645	p=0.1155	F (1, 27) = 0.001065	p=0.9742			
		Age: F (1, 27) = 4.535	p=0.0425					
Body Weight	All	Age: F (1,114) = 352.6	P<0.0001	F (1,114) = 29.94	P<0.0001	Age	Male Female	p<0.0001 p<0.0001
		Sex: F (1,114) = 158.5	P<0.0001			Sex	Young Aged	p<0.0001 p<0.0001
		Pearson's R coefficient	p-value					
Body Weight & Mechanical Allodynia	Males – Day 1	R ² = 0.270	p=0.0132					
	Males – Day 2	R ² = 0.015	p=0.5844					
	Females – Day 1	R ² = 0.154	p=0.107					
	Females – Day 2	R ² = 0.009	p=0.7035					

Key: *no individual timepoints were significant in *post hoc* analysis

Supplemental Table 3.2.

Statistical values for analyses performed within Figure 2. All datasets were analyzed using Ordinary Two-Way ANOVA with Bonferroni's *post hoc*.

Dataset	Main Effect		Interactions		Multiple Comparisons		
	F (DFn, DFd)	p-value	F (DFn, DFd)	p-value	Effect	Groups	p-value
IENF Density	Sex: F (1, 9) = 5.768	p=0.0398	F (1, 9) = 1.744	p=0.0213	Sex	Young	p=0.7276
	Age: F (1, 9) = 6.396	p=0.0323				Aged	p=0.0463
					Age	Male	p=0.0399
						Female	p=6744
IENF Length	Sex: F (1, 9) = 7.677	p=0.0217	F (1, 9) = 0.2302	p=0.6427	Sex	Young	p=0.1033
	Age: F (1, 9) = 1.649	p=0.2312				Aged	p=0.2395

Supplemental Table 3.

Statistical values for analyses performed within Figure 3. All datasets were analyzed with Ordinary Two-Way ANOVA with Bonferroni's *post hoc*.

Dataset		Main Effect		Interactions		Multiple Comparisons		
		F (DFn, DFd)	p-value	F (DFn, DFd)	p-value	Effect	Groups	p-value
Relative Frequency of Neurons (%)	FG+ IB4+	Sex: F (1, 8) = 16.74	p=0.0035	F (1, 8) = 0.3136	p=0.5908	Sex	Young	p=0.0742
		Age: F (1, 8) = 15.04	p=0.0047				Aged	p=0.0221
						Age	Male	p=0.094
	FG+ NF200+	Sex: F (1, 8) = 3.833	p=0.0860	F (1, 8) = 0.07067	p=0.7971		Female	p=0.0277
	FG+ IB4- NF200-	Sex: F (1, 8) = 0.03537	p=0.8555	F (1, 8) = 0.00081	p=0.9779			
		Age: F (1, 8) = 0.1229	p=0.7350					
Number of IB4+ and NF200+ neurons	Small (<400µm ²)	Sex: F (1, 8) = 9.161	p=0.0164	F (1, 8) = 0.7478	p=0.4123	Sex	Young	p=0.05
		Age: F (1, 8) = 0.1477	p=0.7107				Aged	p=0.3297
	Medium (<800µm ²)	Sex: F (1, 8) = 2.654	p=0.1419	F (1, 8) = 0.4874	p=0.5049			
	Large (>800 µm ²)	Age: F (1, 8) = 0.5669	p=0.4731	F (1, 8) = 0.6375	p=0.4477			
		Sex: F (1, 8) = 1.301	p=0.2870					
		Age: F (1, 8) = 4.426	p=0.0685					
Relative Frequency of IB4+ and NF200+ neurons	Small (<400µm ²)	Sex: F (1, 8) = 7.810	p=0.0234	F (1, 8) = 0.1546	p=0.7045	Sex	Young	p=0.1084
		Age: F (1, 8) = 3.726	p=0.0897				Aged	p=0.2558
	Medium (<800µm ²)	Sex: F (1, 8) = 0.006956	p=0.9356	F (1, 8) = 0.003228	p=0.9561	Age	Male	p=0.2093
	Large (>800 µm ²)	Age: F (1, 8) = 6.142	p=0.0382	F (1, 8) = 0.03253	p=0.8614	Age	Female	p=0.2347
		Sex: F (1, 8) = 1.175	p=0.3100				Male	p=0.1837
		Age: F (1, 8) = 6.389	p=0.0354				Female	p=0.2711
Relative Frequency of IB4+ neurons	Small (<400µm ²)	Sex: F (1, 8) = 10.56	p=0.0117	F (1, 8) = 1.962	p=0.1989	Sex	Young	p=0.0221
		Age: F (1, 8) = 2.642	p=0.1428				Aged	p=0.4547
	Medium (<800µm ²)	Sex: F (1, 8) = 7.616	p=0.0247	F (1, 8) = 1.789	p=0.2179	Sex	Young	p=0.04
	Large (>800 µm ²)	Age: F (1, 8) = 2.060	p=0.1892	F (1, 8) = 0.03821	p=0.8499		Aged	p=0.6881
		Sex: F (1, 8) = 3.438	p=0.1008					
		Age: F (1, 8) = 5.028	p=0.0552					
Relative Frequency of NF200+ neurons	Small (<400µm ²)	Sex: F (1, 8) = 3.438	p=0.1008	F (1, 8) = 0.03821	p=0.8499			
		Age: F (1, 8) = 5.028	p=0.0552					
	Medium (<800µm ²)	Sex: F (1, 8) = 0.5757	p=0.4698	F (1, 8) = 0.02203	p=0.8857			
	Large (>800 µm ²)	Age: F (1, 8) = 4.988	p=0.0560	F (1, 8) = 0.001229	p=0.9729			
		Sex: F (1, 8) = 1.274	p=0.2917					
		Age: F (1, 8) = 5.202	p=0.0520					

Supplemental Table 3.4.

Statistical values for analyses performed within Figure 4. All datasets were analyzed with Two-Way ANOVA with Bonferroni's *post hoc*.

Dataset		Main Effect		Interactions		Multiple Comparisons		
		F (DFn, DFd)	p-value	F (DFn, DFd)	p-value	Effect	Groups	p-value
Naïve Paw Thickness	Epidermis Thickness	Sex: F (1, 12) = 5.268 Age: F (1, 12) = 1.201	p=0.0405 p=0.2947	F (1, 12) = 3.078	p=0.1048	Sex	Young Aged	p>0.9999 p=0.0285
	Stratum Corneum	Sex: F (1, 12) = 1.355 Age: F (1, 12) = 0.2740	p=0.2671 p=0.6102	F (1, 12) = 3.158	p=0.1009			
	Stratum Lucidum + Granulosum	Sex: F (1, 12) = 7.219 Age: F (1, 12) = 9.863	p=0.0198 p=0.0085	F (1, 12) = 0.7110	p=0.4156	Sex	Young Aged	p=0.4337 p=0.0562
	Stratum Spinosum + Basale	Sex: F (1, 12) = 6.599 Age: F (1, 12) = 7.796	p=0.0246 p=0.0163	F (1, 12) = 0.1729	p=0.6849	Sex	Young Aged	p=0.3076 p=0.113
Incised Paw Thickness	Epidermis Thickness	Sex: F (1, 11) = 0.6402 Age: F (1, 11) = 9.389	p=0.4406 p=0.0108	F (1, 11) = 0.9052	p=0.3618	Age	Male Female	p=0.0387 p=0.2965
	Stratum Corneum	Sex: F (1, 11) = 0.5959 Age: F (1, 11) = 0.2120	p=0.4564 p=0.6542	F (1, 11) = 1.239	p=0.2893			
	Stratum Lucidum + Granulosum	Sex: F (1, 11) = 0.1617 Age: F (1, 11) = 5.019	p=0.6970 p=0.0518	F (1, 11) = 0.7883	p=0.3977			
	Stratum Spinosum + Basale	Sex: F (1, 11) = 0.6757 Age: F (1, 11) = 5.763	p=0.4323 p=0.0399	F (1, 11) = 0.1729	p=0.1721	Age	Male Female	p=0.0386 p>0.9999
Paw Temperature	Male	Time: F (3.505, 52.57) = 12.84 Age: F (1, 15) = 16.37	p<0.0001 p=0.0108	F (10, 150) = 2.022	p=0.0347	Time	BL 1 2 3 4 5 6 7 8 9 10	p>0.9999 p=0.0235 p=0.0624 p>0.9999 p=0.0557 p>0.9999 p=0.4465 p=0.1944 p>0.9999 p>0.9999 p>0.9999
	Female	Time: F (4.468, 44.68) = 3.437 Age: F (1, 10) = 1.875	p=0.0128 p=0.2008	F (10, 100) = 1.663	p=0.1001			
	Effect Size	Sex: F (1, 25) = 5.388 Age: F (1, 25) = 10.47	p=0.0287 p=0.0034	F (1, 25) = 6.795	p=0.0152	Sex	Young Aged	p>0.9999 p=0.0122
						Age	Male Female	p=0.0007 p>0.9999

Supplemental Table3. 5.

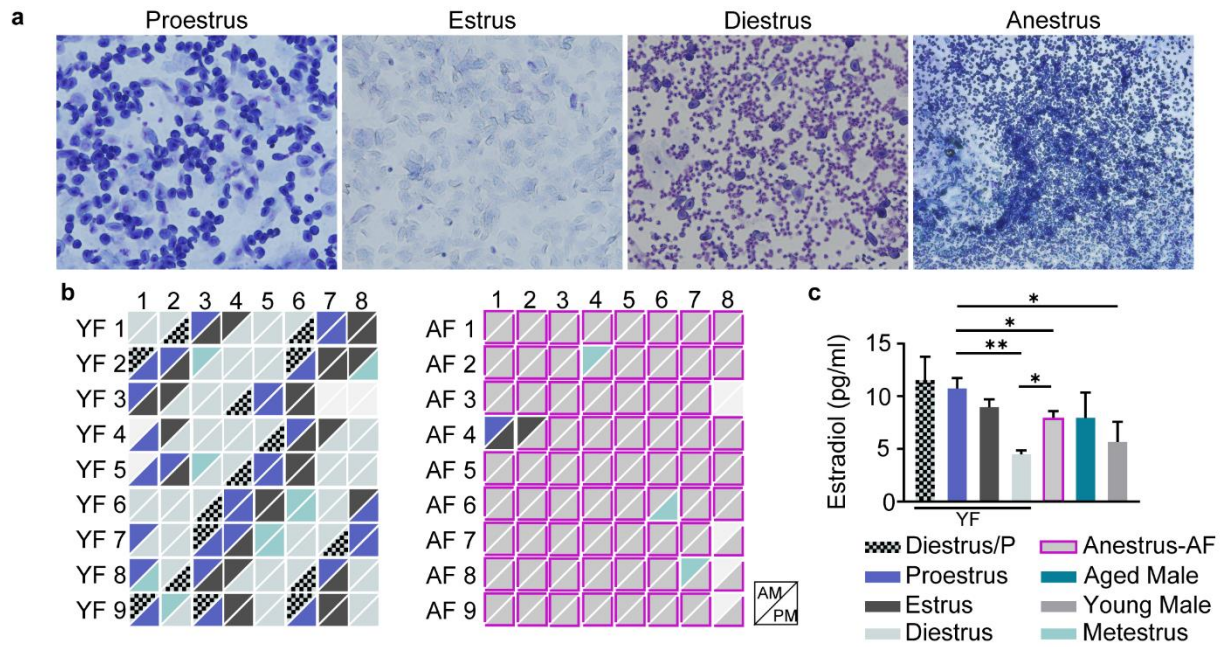
Statistical values for analyses performed within Figure 5. All datasets were analyzed with Two-Way ANOVA with Bonferroni's *post hoc*.

Dataset		Main Effect		Interactions		Multiple Comparisons		
		F (DFn, DFd)	<i>p</i> -value	F (DFn, DFd)	<i>p</i> -value	Effect	Groups	<i>p</i> -value
Naïve Paw Macrophages	Epidermis	Sex: F (1, 8) = 8.948	<i>p</i>=0.0173	F (1, 8) = 10.91	<i>p</i>=0.0108	Sex	Young	<i>p</i> >0.9999
		Age: F (1, 8) = 14.07	<i>p</i>=0.0056				Aged	<i>p</i>=0.0128
	Dermis	Sex: F (1, 8) = 0.2556	<i>p</i> =0.876	F (1, 8) = 0.1362	<i>p</i> =0.7216	Age	Male	<i>p</i>=0.0064
Age: F (1, 8) = 6.965		<i>p</i>=0.0298	Female				<i>p</i> >0.9999	
Total	Sex: F (1, 8) = 0.7682	<i>p</i> =0.4063	F (1, 8) = 1.273	<i>p</i> =0.2919		Male	<i>p</i> =0.2943	
		Age: F (1, 8) = 1.733	<i>p</i> =0.2245			Female	<i>p</i> =0.1322	
Incised Paw Macrophages	Epidermis	Sex: F (1, 8) = 0.004819	<i>p</i> =0.9464	F (1, 8) = 4.309	<i>p</i> =0.0716	Sex	Young	<i>p</i> =0.1499
		Age: F (1, 8) = 1.456	<i>p</i> =0.2621				Aged	<i>p</i> =0.1529
	Dermis	Sex: F (1, 8) = 8.321	<i>p</i>=0.0204	F (1, 8) = 7.942e-005	<i>p</i> =0.9931	Age	Male	<i>p</i> =0.0638
Age: F (1, 8) = 0.4709		<i>p</i> =0.5120	Aged				<i>p</i> =0.3964	
Total	Sex: F (1, 8) = 7.989	<i>p</i>=0.0223	F (1, 8) = 0.7095	<i>p</i> =0.4241				
		Age: F (1, 8) = 1.789	<i>p</i> =0.2179					
Naïve Paw vs Incised Paw Macrophages	Epidermis	Surgery: F (1, 16) = 45.16	<i>p</i><0.0001	F (3, 16) = 2.060	<i>p</i> =0.1460	Surgery	Young Male	<i>p</i>=0.0131
		Age/Sex: F (3, 16) = 2.839	<i>p</i> =0.0710				Aged Male	<i>p</i> =0.0732
	Dermis	Surgery: F (1, 16) = 138.7	<i>p</i><0.0001	F (3, 16) = 2.060	<i>p</i> =0.0797	Surgery	Young Female	<i>p</i>=0.0003
		Age/Sex: F (3, 16) = 3.097	<i>p</i> =0.0565				Aged Female	<i>p</i> =0.236
							Young Male	<i>p</i>=0.0013
							Aged Male	<i>p</i>=0.0019
	Total	Surgery: F (1, 16) = 155.3	<i>p</i><0.0001	F (3, 16) = 3.393	<i>p</i>=0.0438	Sex	Young Female	<i>p</i><0.0001
		Age/Sex: F (3, 16) = 3.449	<i>p</i>=0.0418				Young	<i>p</i> >0.9999
							Aged	<i>p</i> >0.9999
							Male	<i>p</i> >0.9999
				Age	Female	<i>p</i> >0.9999		
				Surgery	Young Male	<i>p</i>=0.0038		
					Aged Male	<i>p</i>=0.0095		
					Young Female	<i>p</i><0.0001		
					Aged Female	<i>p</i>=0.0001		

Supplemental Table 3.6.

Statistical values for analyses performed within Supplementary Figure 1. All datasets were analyzed with Student's t test.

Dataset	Comparisons		t, df	p-value
Serum Estradiol	Diestrus/Proestrus	Proestrus	0.3851, 9	$p=0.7091$
	Proestrus	Estrus	1.017, 9	$p=0.3356$
	Proestrus	Diestrus	4.263, 10	$p=0.0017$
	Proestrus	Anestrus	2.307, 22	$p=0.0308$
	Proestrus	Aged Male	1.236, 11	$p=0.2422$
	Proestrus	Young Male	2.601, 11	$p=0.0247$
	Diestrus	Anestrus	2.246, 18	$p=0.0375$
	Anestrus	Aged Male	0.05298, 19	$p=0.9583$
	Anestrus	Young Male	1.324, 19	$p=0.2012$



Supplementary Fig. 3.1. Estrus cycle is interrupted in aged females. **a**, Young female vaginal samples alternate between proestrus, predominance of nucleated epithelia cells, estrus, predominance of anucleated cornified epithelial cells, metestrus (not represented, all cells present) and diestrus, predominantly leucocytes. Meanwhile, aged female vaginal samples are chronically on anestrus, where leucocytes are predominantly present. Scale bar = 100 μ m. **b**, Sequential estrus phase of young and aged FBN females within 8 days. **c**, Serum levels of estradiol in young females during different phases of the estrus cycle, as well as aged females and male rats. $n=4$ for all groups. Data are represented as mean \pm SEM. Two-sided student t-test was used. * $p<0.05$, ** $p<0.01$.