

Supplementary Material

Unique nicotinic responses are present in distinct subtypes of mouse medial prefrontal layer V pyramidal neurons

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Supplementary Table S1. Data reporting and statistical comparisons.

Measure	Group	Sample Size	Mean	SD	Effect Tested	Test Used	F	P
Figure 1								
Proportion of neurons showing each nicotinic response type	Male	57 (total)	N/A	N/A	Male versus Female	Chi-square	0.5496	0.7597
	Female	63 (total)	N/A	N/A				
Figure 3								
Current Amplitude (pA)	Male: $\alpha 7$	29	328.0	197.0	nAChR response type	Two-Way ANOVA	16.29	<0.0001
	Male: $\alpha 7/\beta 2^*$	13	187.5	51.7				
	Male: $\beta 2^*$	15	133.6	52.8				
	Female: $\alpha 7$	34	271.3	182.8	Males versus Female	Two-Way ANOVA	1.14	0.2884
	Female: $\alpha 7/\beta 2^*$	14	171.2	65.6				
	Female: $\beta 2^*$	13	114.1	60.8				
Note: Outliers removed: 1 from Female: $\alpha 7$ and 1 from Female: $\alpha 7\beta 2^*$								
Net Charge of Response (pC)	Male: $\alpha 7$	29	77426.5	56105.8	nAChR response type	Two-Way ANOVA	64.61	<0.0001
	Male: $\alpha 7/\beta 2^*$	13	114261.2	38743.5				
	Male: $\beta 2^*$	15	364789.1	163277.4				
	Female: $\alpha 7$	35	84973.2	67751.9	Males versus Female	Two-Way ANOVA	1.84	0.1775
	Female: $\alpha 7/\beta 2^*$	15	145350.9	60044..2				
	Female: $\beta 2^*$	13	254777.2	133967.3				
Time to Peak (ms)	Male: $\alpha 7$	29	39.2	22.0	nAChR response type	Two-Way ANOVA	196.10	<0.0001
	Male: $\alpha 7/\beta 2^*$	13	37.9	22.1				
	Male: $\beta 2^*$	15	540.1	216.3				
	Female: $\alpha 7$	35	42.9	21.9	Males versus Female	Two-Way ANOVA	0.37	0.5453
	Female: $\alpha 7/\beta 2^*$	15	36.5	22.8				
	Female: $\beta 2^*$	13	497.7	245.8				
Rise Tau (ms)	Male: $\alpha 7$	29	21.2	15.1	nAChR response type	Two-Way ANOVA	84.42	<0.0001
	Male: $\alpha 7/\beta 2^*$	13	23.4	9.8				
	Male: $\beta 2^*$	15	128.7	68.8				
	Female: $\alpha 7$	35	25.7	18.2	Males versus Female	Two-Way ANOVA	0.66	0.4168
	Female: $\alpha 7/\beta 2^*$	15	27.5	23.4				
	Female: $\beta 2^*$	13	104.2	50.2				
1 st Decay Tau (ms)	Male: $\alpha 7$	29	69.2	34.4	nAChR response type	Two-Way ANOVA	418.80	<0.0001
	Male: $\alpha 7/\beta 2^*$	13	194.5	190.8				
	Male: $\beta 2^*$	15	2558.4	694.4				
	Female: $\alpha 7$	35	94.6	54.9	Males versus Female	Two-Way ANOVA	0.51	0.4747
	Female: $\alpha 7/\beta 2^*$	15	465.6	509.4				
	Female: $\beta 2^*$	13	2112.3	504.5				

Figure 3, continued

Measure	Group	Sample Size	Mean	SD	Effect Tested	Test Used	F	P
2 nd Decay Tau (ms)	Male: $\alpha 7$	29	378.0	228.1	nAChR response type	Two-Way ANOVA	361.60	<0.0001
	Male: $\alpha 7/\beta 2^*$	13	1884.7	706.1				
	Male: $\beta 2^*$	15	4336.1	885.6				
	Female: $\alpha 7$	35	465.8	230.0	Males versus Female	Two-Way ANOVA	0.74	0.3915
	Female: $\alpha 7/\beta 2^*$	15	2331.4	995.1				
	Female: $\beta 2^*$	13	3505.3	616.3				

Figure 4 and related measures

Resting Membrane Potential (mV)	Male: $\alpha 7$	29	-72.5	2.6	nAChR response type	Two-Way ANOVA	2.33	0.1019
	Male: $\alpha 7/\beta 2^*$	13	-71.2	1.1				
	Male: $\beta 2^*$	15	-71.9	4.6				
	Female: $\alpha 7$	35	-71.7	2.1	Males versus Female	Two-Way ANOVA	0.25	0.6189
	Female: $\alpha 7/\beta 2^*$	15	-71.2	1.7				
	Female: $\beta 2^*$	13	-73.4	3.0				
Input Resistance (M Ω)	Male: $\alpha 7$	29	236.3	71.8	nAChR response type	Two-Way ANOVA	29.26	<0.0001
	Male: $\alpha 7/\beta 2^*$	13	195.4	82.9				
	Male: $\beta 2^*$	15	324.3	39.4				
	Female: $\alpha 7$	35	230.9	79.4	Males versus Female	Two-Way ANOVA	0.74	0.3902
	Female: $\alpha 7/\beta 2^*$	15	204.0	76.1				
	Female: $\beta 2^*$	13	358.9	84.5				
ISI Ratio	Male: $\alpha 7$	29	0.19	0.10	nAChR response type	Two-Way ANOVA	46.71	>0.0001
	Male: $\alpha 7/\beta 2^*$	13	0.20	0.06				
	Male: $\beta 2^*$	14	0.45	0.15				
	Female: $\alpha 7$	35	0.19	0.08	Males versus Female	Two-Way ANOVA	2.86	0.0934
	Female: $\alpha 7/\beta 2^*$	15	0.20	0.10				
	Female: $\beta 2^*$	13	0.35	0.08				
Note: Outliers removed: 1 from Male: $\beta 2^*$								
Rheobase (pA)	Male: $\alpha 7$	29	44.1	18.5	nAChR response type	Two-Way ANOVA	5.10	0.0076
	Male: $\alpha 7/\beta 2^*$	13	57.7	23.6				
	Male: $\beta 2^*$	15	42.0	10.0				
	Female: $\alpha 7$	35	40.1	15.4	Males versus Female	Two-Way ANOVA	5.22	0.0242
	Female: $\alpha 7/\beta 2^*$	14	46.8	23.3				
	Female: $\beta 2^*$	13	32.7	15.9				
Note: Outliers removed: 1 from Female: $\alpha 7/\beta 2^*$								
Sag Ratio	Male: $\alpha 7$	29	0.10	0.09	nAChR response type	Two-Way ANOVA	2.67	0.0736
	Male: $\alpha 7/\beta 2^*$	13	0.15	0.08				
	Male: $\beta 2^*$	15	0.10	0.04				
	Female: $\alpha 7$	35	0.12	0.10	Males versus Female	Two-Way ANOVA	0.0059	0.9390
	Female: $\alpha 7/\beta 2^*$	15	0.13	0.08				
	Female: $\beta 2^*$	13	0.09	0.04				

Figure 4, continued

Measure	Effect Tested	Test Used	F	P
Input / Output Curve – Male data only	nAChR response type	RM Two-Way ANOVA	0.29	0.7495
	Current injected		136.10	<0.0001
	Interaction		14.92	<0.0001
	Matching		7.71	<0.0001
Input / Output Curve – Female data only	nAChR response type	RM Two-Way ANOVA	0.26	0.7715
	Current injected		82.62	<0.0001
	Interaction		8.84	<0.0001
	Matching		6.72	<0.0001
Input / Output Curve – BF ($\alpha 7$) neurons only	Sex (Male versus Female)	RM Two-Way ANOVA	2.08	0.1542
	Current Injected		187.30	<0.0001
	Interaction		0.6965	0.7124
	Matching		4.9290	<0.0001
Input / Output Curve – BF ($\alpha 7/\beta 2^*$) neurons only	Sex (Male versus Female)	RM Two-Way ANOVA	0.16	0.6969
	Current Injected		145.10	<0.0001
	Interaction		0.12	0.9992
	Matching		16.55	<0.0001
Input / Output Curve – RF ($\beta 2^*$) neurons only	Sex (Male versus Female)	RM Two-Way ANOVA	1.51	0.2298
	Current Injected		30.45	<0.0001
	Interaction		0.79	0.6239
	Matching		6.38	<0.0001
AHP Amplitude – Male data only	nAChR response type	RM Two-Way ANOVA	2.35	0.1060
	Number of current pulses		221.60	<0.0001
	Interaction		6.66	<0.0001
	Matching		16.77	<0.0001
AHP Amplitude – Female data only	nAChR response type	RM Two-Way ANOVA	0.31	0.7365
	Number of current pulses		208.40	<0.0001
	Interaction		2.16	0.0200
	Matching		17.22	<0.0001
AHP Amplitude – BF ($\alpha 7$) neurons only	Sex (Male versus Female)	RM Two-Way ANOVA	2.47	0.1217
	Current Injected		287.80	<0.0001
	Interaction		1.27	0.2761
	Matching		18.25	<0.0001
AHP Amplitude – BF ($\alpha 7/\beta 2^*$) neurons only	Sex (Male versus Female)	RM Two-Way ANOVA	0.078	0.7823
	Current Injected		155.40	<0.0001
	Interaction		0.3732	0.8663
	Matching		16.58	<0.0001

Figure 4, continued

Measure	Effect Tested	Test Used	F	P
AHP Amplitude – BF ($\alpha 7/\beta 2^*$) neurons only	Sex (Male versus Female)	RM Two-Way ANOVA	3.02	0.0953
	Current Injected		76.04	<0.0001
	Interaction		1.87	0.1042
	Matching		13.84	<0.0001

Figure 5

Measure	Group	Sample Size	Mean	SD	Effect Tested	Test Used	F	P
EPSC Frequency (Hz)	Male: $\alpha 7$	29	5.63	2.34	nAChR response type	Two-Way ANOVA	13.41	<0.0001
	Male: $\alpha 7/\beta 2^*$	13	5.34	2.51				
	Male: $\beta 2^*$	15	3.33	0.86				
	Female: $\alpha 7$	35	4.85	2.36	Males versus Female	Two-Way ANOVA	0.35	0.5579
	Female: $\alpha 7/\beta 2^*$	15	6.19	2.85				
	Female: $\beta 2^*$	12	2.49	1.33				
Note: Outliers removed: 1 from Female: $\beta 2^*$								
EPSC Amplitude (pA)	Male: $\alpha 7$	29	14.14	3.30	nAChR response type	Two-Way ANOVA	10.31	<0.0001
	Male: $\alpha 7/\beta 2^*$	13	11.96	3.40				
	Male: $\beta 2^*$	15	11.24	2.75				
	Female: $\alpha 7$	34	11.13	3.13	Males versus Female	Two-Way ANOVA	20.34	<0.0001
	Female: $\alpha 7/\beta 2^*$	15	10.26	2.46				
	Female: $\beta 2^*$	12	7.84	2.22				
Note: Outliers removed: 1 from Female: $\alpha 7$ and 1 from Female: $\beta 2^*$								
EPSC Net Charge (pC)	Male: $\alpha 7$	29	142.32	40.80	nAChR response type	Two-Way ANOVA	17.26	<0.0001
	Male: $\alpha 7/\beta 2^*$	13	111.63	39.46				
	Male: $\beta 2^*$	15	82.39	51.06				
	Female: $\alpha 7$	35	95.72	32.12	Males versus Female	Two-Way ANOVA	19.91	<0.0001
	Female: $\alpha 7/\beta 2^*$	15	85.87	20.45				
	Female: $\beta 2^*$	12	58.23	22.50				
Note: Outliers removed: 1 from Female: $\beta 2^*$								

Figure 7

Measure	Effect Tested	Test Used	F	P
Apical Dendrite Length - ♂	nAChR response type	Two-Way ANOVA	11.99	<0.0001
	Distance from soma		15.78	<0.0001
	Interaction		1.51	0.0160
Basal Dendrite Length - ♂	nAChR response type	Two-Way ANOVA	41.55	<0.0001
	Distance from soma		105.3	<0.0001
	Interaction		3.63	<0.0001
Apical Dendrite Length - ♀	nAChR response type	Two-Way ANOVA	16.52	<0.0001
	Distance from soma		8.94	<0.0001
	Interaction		0.89	0.6755
Basal Dendrite Length - ♀	nAChR response type	Two-Way ANOVA	8.82	0.0002
	Distance from soma		94.49	<0.0001
	Interaction		3.37	<0.0001
Apical Dendrite Diameter - ♂	nAChR response type	Two-Way ANOVA	20.81	<0.0001
	Distance from soma		30.41	<0.0001
	Interaction		0.76	0.8906
Basal Dendrite Diameter - ♂	nAChR response type	Two-Way ANOVA	16.65	<0.0001
	Distance from soma		37.68	<0.0001
	Interaction		0.89	0.6533
Apical Dendrite Diameter - ♀	nAChR response type	Two-Way ANOVA	53.13	<0.0001
	Distance from soma		18.76	<0.0001
	Interaction		0.65	0.9570
Basal Dendrite Diameter - ♀	nAChR response type	Two-Way ANOVA	1.60	0.2025
	Distance from soma		55.61	<0.0001
	Interaction		1.27	0.1458
Apical Dendrite Volume - ♂	nAChR response type	Two-Way ANOVA	14.19	<0.0001
	Distance from soma		14.55	<0.0001
	Interaction		1.06	0.3633
Basal Dendrite Volume - ♂	nAChR response type	Two-Way ANOVA	18.79	<0.0001
	Distance from soma		44.74	<0.0001
	Interaction		2.47	<0.0001
Apical Dendrite Volume - ♀	nAChR response type	Two-Way ANOVA	65.19	<0.0001
	Distance from soma		10.36	<0.0001
	Interaction		0.76	0.8605
Basal Dendrite Volume - ♀	nAChR response type	Two-Way ANOVA	25.46	<0.0001
	Distance from soma		112.00	<0.0001
	Interaction		6.80	<0.0001

Figure 8

Measure	Effect Tested	Test Used	F	P
Apical dendrite volume – BF ($\alpha 7$)	Males versus Females	Two-Way ANOVA	110.20	<0.0001
	Distance from soma		12.25	<0.0001
	Interaction		1.91	0.0054
Basal dendrite volume – BF ($\alpha 7$)	Males versus Females	Two-Way ANOVA	5.57	0.0188
	Distance from soma		150.00	<0.0001
	Interaction		5.58	<0.0001
Apical dendrite volume – BF ($\alpha 7/\beta 2^*$)	Males versus Females	Two-Way ANOVA	3.69	0.0556
	Distance from soma		8.69	<0.0001
	Interaction		0.63	0.9143
Basal dendrite volume – BF ($\alpha 7/\beta 2^*$)	Males versus Females	Two-Way ANOVA	0.028	0.8666
	Distance from soma		25.94	<0.0001
	Interaction		0.22	0.9998
Apical dendrite volume – RF ($\beta 2^*$)	Males versus Females	Two-Way ANOVA	4.77	0.0297
	Distance from soma		9.72	<0.0001
	Interaction		0.89	0.6239
Basal dendrite volume – BF ($\beta 2^*$)	Males versus Females	Two-Way ANOVA	11.82	0.0007
	Distance from soma		31.29	<0.0001
	Interaction		0.87	0.6130