APPENDIX 2: Full Collection of Data for the Backbone Ester Mutation at L119 in the α4β2 and Muscle-Type Nicotinic Acetylcholine Receptors (nAChRs)

This appendix compiles all of the data from Chapters 2-4 concerning the backbone ester mutation at L119 in the complementary subunit of α4β2 and the muscle-type nAChRs.

Key observations:

1. The largest fold shift is seen for (−)-Cytisine at the $(\alpha4)_2(\beta2)_3$ receptor despite its low efficacy for this receptor (~0.03 relative to ACh).
2. Varenicline is unaffected by this mutation at both stoichiometries of α4β2.
3. Epibatidine is sensitive to the backbone ester mutation in α4β2 but not in the muscle-type receptor.
4. In general, larger fold-shifts are seen in the muscle-type receptor than in α4β2.
5. The α1G153K mutation has little impact on the L119 interaction.
6. The weak agonist choline (which does not contain the hydrogen bond acceptor of the pharmacophore) is unaffected by the backbone ester mutation, but the nicotine analog *S*-MPP gives a large gain-of-function in the α4β2 receptor.

Table A2.1. EC_{50} values, Hill coefficients, and relative efficacies for the muscle-type receptor. Errors are standard error of the mean. Mutations identified as "Leu" represent recovery of the wild-type receptor by nonsense suppression.

Agonist	Mutation	EC_{50} (nM)	Fold-Shift	Hill (n_H)
	α1₂β1γδ			
ACh	α1β1γδ	16000 ± 300		1.3 ± 0.1
	α1β1γ(L119Leu)δ(L121Leu)	16000 ± 500		1.5 ± 0.1
	α1β1γ(L119Lah)δ(L121Lah)	230000 ± 6000	14	1.5 ± 0.1
	α1₂β1(L9'S)γδ			
ACh	α1β1(L9'S)γδ	610 ± 40		1.4 ± 0.1
	α1β1(L9'S)γ(L119Leu)δ(L121Leu)	310 ± 20		1.5 ± 0.1
	α1β1(L9'S)γ(L119Lah)δ(L121Lah)	9100 ± 700	29	1.6 ± 0.2
Ch	α1β1(L9'S)γδ	840000 ± 20000		1.6 ± 0.1
	α1β1(L9'S)γ(L119Leu)δ(L121Leu)	780000 ± 30000		1.7 ± 0.1
	α1β1(L9'S)γ(L119Lah)δ(L121Lah)	1000000 ± 50	1.3	1.8 ± 0.1
S-Nic	α1β1(L9'S)γδ	22000 ± 800		1.6 ± 0.1
	α1β1(L9'S)γ(L119Leu)δ(L121Leu)	23000 ± 700		1.7 ± 0.1
	α1β1(L9'S)γ(L119Lah)δ(L121Lah)	230000 ± 30000	10	2.2 ± 0.5
(±)-Epi	α1β1(L9'S)γδ	320 ± 20		1.5 ± 0.1
	α1β1(L9'S)γ(L119Leu)δ(L121Leu)	400 ± 20		1.5 ± 0.1
	α1β1(L9'S)γ(L119Lah)δ(L121Lah)	520 ± 30	1.3	1.6 ± 0.1
	α1(G153K)₂β1(L9'S)γδ			
ACh	α1(G153K)β1(L19'S)γδ	7.2 ± 0.7		1.3 ± 0.1
	α1(G153K)β1(L9'S)γ(L119Leu)δ(L121Leu)	7.6 ± 0.7		1.8 ± 0.3
	α1(G153K)β1(L9'S)γ(L119Lah)δ(L121Lah)	180 ± 20	24	1.3 ± 0.1
Ch	α1(G153K)β1(L19'S)γδ	30000 ± 2000		1.0 ± 0.1
	α1(G153K)β1(L9'S)γ(L119Leu)δ(L121Leu)	27000 ± 2000		1.1 ± 0.1
	α1(G153K)β1(L9'S)γ(L119Lah)δ(L121Lah)	68000 ± 3000	2.5	1.3 ± 0.1
S-Nic	α1(G153K)β1(L19'S)γδ	320 ± 30		1.4 ± 0.2
	α1(G153K)β1(L9'S)γ(L119Leu)δ(L121Leu)	360 ± 40		0.95 ± 0.1
	α1(G153K)β1(L9'S)γ(L119Lah)δ(L121Lah)	6500 ± 500	18	1.3 ± 0.1
(±)-Epi	α1(G153K)β1(L19'S)γδ	4.3 ± 0.5		0.77 ± 0.5
	α1(G153K)β1(L9'S)γ(L119Leu)δ(L121Leu)	2.3 ± 0.4		0.74 ± 0.1
	α1(G153K)β1(L9'S)γ(L119Lah)δ(L121Lah)	9.6 ± 0.4	4.2	1.1 ± 0.1

Table A2.2. EC_{50} values, Hill coefficients, and relative efficacies for the α4β2 receptor. All studies gave current values at +70 mV (normalized to −110 mV) of ≤0.08 or >0.2, confirming the A2B3 or A3B2 stoichiometry, respectively. Errors are standard error of the mean. Mutations identified as "Leu" represent recovery of the wild-type receptor by nonsense suppression. The relative efficacy is the ratio of the I_{max} of a saturating concentration of agonist / I_{max} of a saturating concentration of ACh. By definition, the relative efficacy of ACh is 1.

Agonist	Mutation	EC_{50} (nM)	Fold-Shift	Hill (n_H)	Relative Efficacy
		$\alpha4(L9'A)_2\beta2_3$			
ACh	α4(L9'A)β2	360 ± 20		1.3 ± 0.1	[1]
	α4(L9'A)β2(L119Leu)	440 ± 20		1.3 ± 0.1	
	α4(L9'A)β2(L119Lah)	3000 ± 100	6.8	1.2 ± 0.1	
CCh	α4(L9'A)β2	7200 ± 80		1.3 ± 0.1	0.50 ± 0.02
	α4(L9'A)β2(L119Leu)	7900 ± 200		1.2 ± 0.1	
	α4(L9'A)β2(L119Lah)	29000 ± 800	3.7	1.2 ± 0.1	
Ch	α4(L9'A)β2	140000 ± 4000		1.6 ± 0.1	0.060 ± 0.01
	α4(L9'A)β2(L119Leu)	135000 ± 20000		1.2 ± 0.1	
	α4(L9'A)β2(L119Lah)	150000 ± 5000	1.1	1.4 ± 0.1	
S-Nic	α4(L9'A)β2	120 ± 5		1.3 ± 0.1	0.27 ± 0.01
	α4(L9'A)β2(L119Leu)	120 ± 3		1.5 ± 0.1	
	α4(L9'A)β2(L119Lah)	800 ± 30	6.7	1.3 ± 0.1	
S-MPP	α4(L9'A)β2	11000 ± 400		1.7 ± 0.1	0.23 ± 0.01
	α4(L9'A)β2(L119Leu)	14000 ± 900		1.5 ± 0.1	
	α4(L9'A)β2(L119Lah)	1100 ± 40	0.1	1.5 ± 0.1	
(R/S)-MPP	α4(L9'A)β2	19000 ± 600		1.8 ± 0.1	0.14 ± 0.02
(±)-Epi	α4(L9'A)β2	0.79 ± 0.04		1.4 ± 0.1	0.47 ± 0.03
	α4(L9'A)β2(L119Leu)	0.58 ± 0.05		1.5 ± 0.1	
	α4(L9'A)β2(L119Lah)	2.9 ± 0.1	5.0	1.3 ± 0.1	
(+)-Epi	α4(L9'A)β2	0.87 ± 0.03		1.5 ± 0.1	0.33 ± 0.01
	α4(L9'A)β2(L119Leu)	0.58 ± 0.04		1.3 ± 0.1	
	α4(L9'A)β2(L119Lah)	2.7 ± 0.1	4.7	1.3 ± 0.1	
(−)-Epi	α4(L9'A)β2	1.1 ± 0.04		1.6 ± 0.1	0.74 ± 0.03
	α4(L9'A)β2(L119Leu)	0.73 ± 0.03		1.4 ± 0.1	
	α4(L9'A)β2(L119Lah)	3.8 ± 0.2	5.2	1.1 ± 0.1	
(+)-Epi-Me	α4(L9'A)β2	8.6 ± 0.5		1.7 ± 0.2	0.50 ± 0.03
	α4(L9'A)β2(L119Leu)	8.9 ± 0.7		1.3 ± 0.1	
	α4(L9'A)β2(L119Lah)	30 ± 2	3.4	1.4 ± 0.1	
(−)-Epi-Me	α4(L9'A)β2	0.42 ± 0.04		1.5 ± 0.1	0.55 ± 0.03
	α4(L9'A)β2(L119Leu)	0.42 ± 0.04		1.6 ± 0.1	
	α4(L9'A)β2(L119Lah)	0.91 ± 0.06	2.2	1.9 ± 0.2	
Cy	α4(L9'A)β2	6.9 ± 0.3		1.4 ± 0.1	0.030 ± 0.01
	α4(L9'A)β2(L119Leu)	8.7 ± 0.4		1.2 ± 0.1	
	α4(L9'A)β2(L119Lah)	540 ± 30	62	0.98 ± 0.1	
Var	α4(L9'A)β2	3.1 ± 0.1		1.4 ± 0.1	0.12 ± 0.02
	α4(L9'A)β2(L119Leu)	2.6 ± 0.2		1.3 ± 0.1	
	α4(L9'A)β2(L119Lah)	4.7 ± 0.2	1.8	1.3 ± 0.1	
		$\alpha4(L9'A)_3\beta2_2$			
ACh	α4(L9'A)β2	26 ± 0.1		1.1 ± 0.1	[1]
	α4(L9'A)β2(L119Leu)	26 ± 0.4		1.6 ± 0.1	
	α4(L9'A)β2(L119Lah)	220 ± 10	8.5	1.2 ± 0.1	
Ch	α4(L9'A)β2	90000 ± 2000		1.4 ± 0.1	0.70 ± 0.06
	α4(L9'A)β2(L119Leu)	76000 ± 200		1.4 ± 0.1	
	α4(L9'A)β2(L119Lah)	74000 ± 2000	1.0	1.5 ± 0.1	
S-Nic	α4(L9'A)β2	12 ± 0.1		1.6 ± 0.1	0.56 ± 0.04
	α4(L9'A)β2(L119Leu)	12 ± 0.1		1.6 ± 0.1	
	α4(L9'A)β2(L119Lah)	67 ± 3	5.6	1.4 ± 0.1	
S-MPP	α4(L9'A)β2	4500 ± 100		1.1 ± 0.1	0.39 ± 0.03
	α4(L9'A)β2(L119Leu)	4200 ± 300		1.6 ± 0.1	
	α4(L9'A)β2(L119Lah)	130 ± 10	0.031	1.2 ± 0.1	
(R/S)-MPP	α4(L9'A)β2	5900 ± 100		1.6 ± 0.1	0.28 ± 0.03
Cy	α4(L9'A)β2	3.1 ± 0.1		1.9 ± 0.1	0.54 ± 0.05
	α4(L9'A)β2(L119Leu)	3.6 ± 0.1		1.9 ± 0.1	
	α4(L9'A)β2(L119Lah)	51 ± 2	14	1.4 ± 0.1	
Var	α4(L9'A)β2	0.95 ± 0.02		1.7 ± 0.1	0.33 ± 0.01
	α4(L9'A)β2(L119Leu)	1.0 ± 0.1		1.5 ± 0.1	
	α4(L9'A)β2(L119Lah)	1.1 ± 0.1	1.1	1.2 ± 0.1	