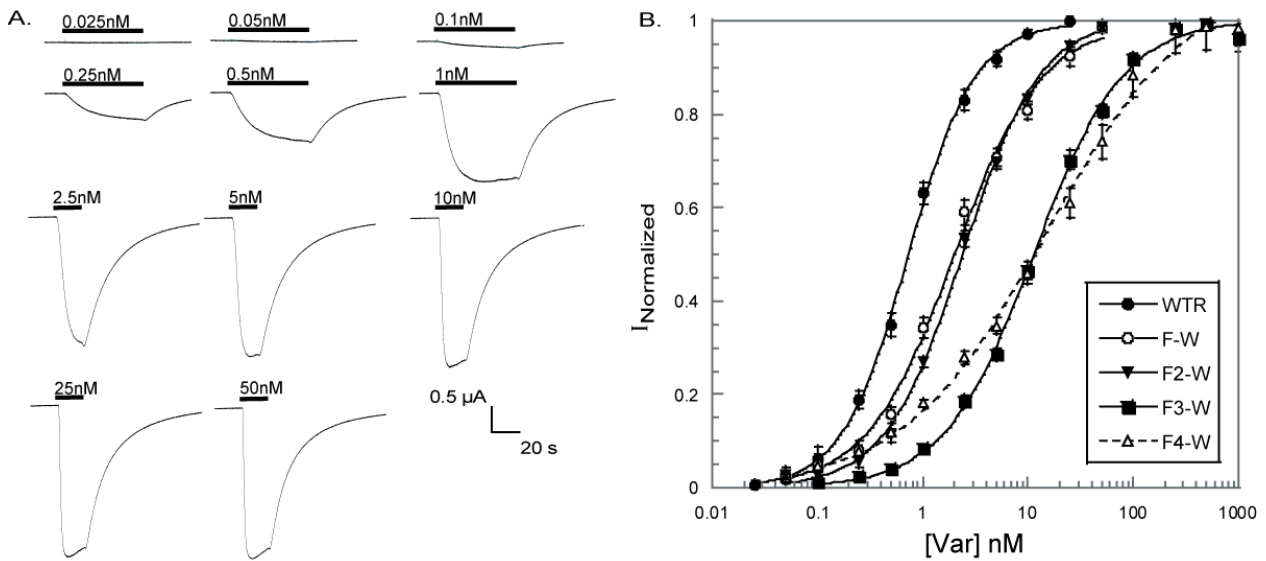


**Supporting Information for:**

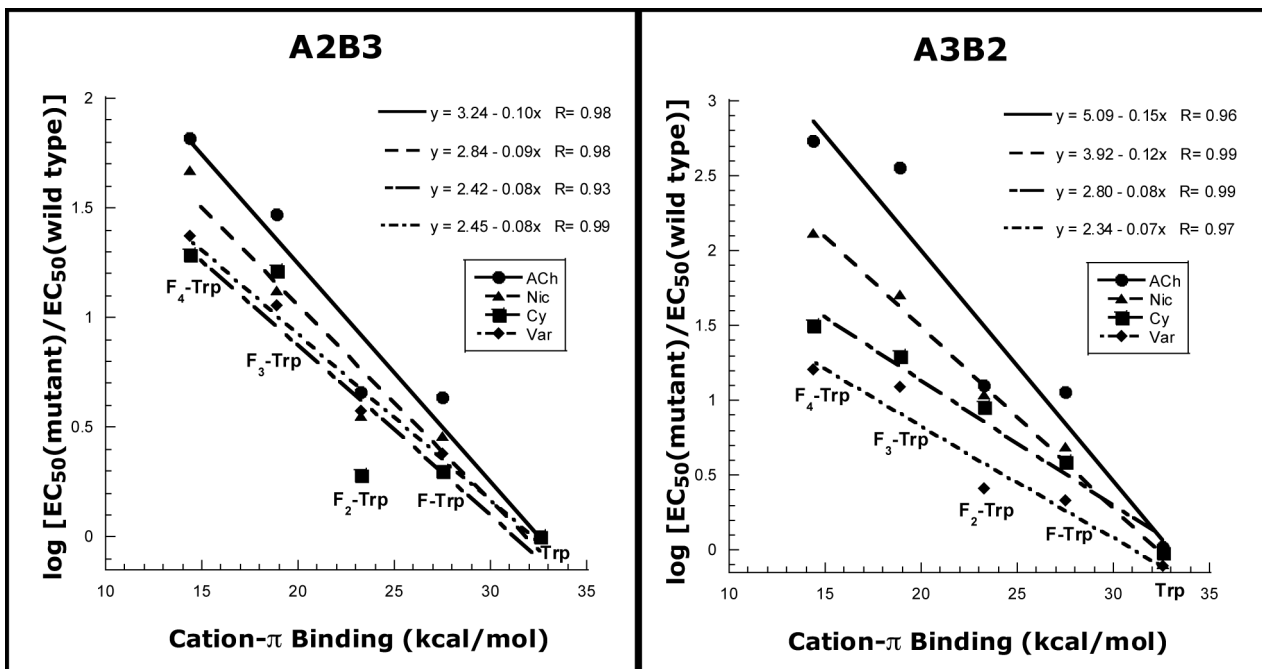
**Variations in Binding Among Several Agonists at Two Stoichiometries of the Neuronal,  $\alpha 4\beta 2$  Nicotinic Receptor**

Ximena Da Silva Tavares,<sup>†</sup> Angela P. Blum,<sup>†</sup> Darren T. Nakamura,<sup>†</sup> Nyssa L. Puskar,<sup>†</sup> Jai A. P. Shanata,<sup>†</sup> Henry A. Lester,<sup>‡</sup> Dennis A. Dougherty<sup>†\*</sup>

**Supporting Figure 1.** A. Representative traces of voltage-clamp current for a wild type recovery experiment at Trp154 of the A3B2 receptor. Bars show application of varenicline at concentrations noted. B. Fit of data for several suppression experiments to the Hill equation for A3B2. Error bars indicate SEM;  $n = 5-8$ .



**Supporting Figure 2.** Fluorination plots for the agonists studied at both stoichiometries of the  $\alpha 4\beta 2$  receptor. Data for nicotine and ACh at A2B3 were previously published by (Xiu et al., 2009) and are reproduced here for comparison purposes. Nic = nicotine; Cy = cytisine; Var = varenicline.



**Supporting Table 1.** EC<sub>50</sub> values (nM), fold shift values, Hill coefficients (n<sub>H</sub>) and current size at +70mV (normalized to current size at -110mV). As described previously (Xiu et al., 2009), we interpret an I<sub>norm</sub> value below 0.10 as corresponding to an essentially pure population of A2B3 receptors and values at or above 0.20 as corresponding to an essentially pure population of A3B2 receptors. Hill coefficients are generally in the typical range, although some heavily fluorinated derivatives show lower than usual values. We have seen this trend in other studies, but we do not have a structural interpretation. All receptor studies reported herein contain a L9'A mutation in the α subunit. Errors are SEM.

A2B3 Receptor						
Agonist	Mutation	EC <sub>50</sub> (nM)	Fold Shift	n <sub>H</sub>	I <sub>norm</sub> (+70mV)	
Cy	Wild type	6.9 ± 0.3	-	1.4 ± 0.1	0.05 ± 0.01	
	α4W154 Trp	11 ± 1	1.6	1.1 ± 0.1	0.03 ± 0.01	
	α4W154 F-Trp	22 ± 1	3.2	1.1 ± 0.1	0.05 ± 0.01	
	α4W154 F <sub>2</sub> -Trp	21 ± 1	3.0	1.1 ± 0.1	0.08 ± 0.01	
	α4W154 F <sub>3</sub> -Trp	180 ± 10	26	1.3 ± 0.1	0.05 ± 0.03	
	α4W154 F <sub>4</sub> -Trp	210 ± 60	31	0.62 ± 0.08	0.04 ± 0.01	
Var	Wild type	2.9 ± 0.1	-	1.4 ± 0.1	0.037 ± 0.007	
	α4W154 Trp	2.4 ± 0.2	0.8	1.2 ± 0.1	0.043 ± 0.005	
	α4W154 F-Trp	5.7 ± 0.2	2.0	1.2 ± 0.1	0.040 ± 0.007	
	α4W154 F <sub>2</sub> -Trp	9.0 ± 0.4	3.1	1.2 ± 0.1	0.05 ± 0.011	
	α4W154 F <sub>3</sub> -Trp	27 ± 1	9.5	1.3 ± 0.1	0.044 ± 0.009	
	α4W154 F <sub>4</sub> -Trp	56 ± 5	20	1.1 ± 0.1	0.033 ± 0.008	

Supporting Table 1, continued

A3B2 Receptor						
Agonist	Mutation	EC <sub>50</sub> (nM)	Fold Shift	n <sub>H</sub>	I <sub>norm</sub> (+70mV)	
ACh	Wild type	23 ± 1	-	1.3 ± 0.1	0.30 ± 0.04	
	α4W154 Trp	24 ± 1	1.0	1.6 ± 0.1	0.27 ± 0.01	
	α4W154 F-Trp	260 ± 10	11	1.2 ± 0.1	0.19 ± 0.01	
	α4W154 F <sub>2</sub> -Trp	290 ± 10	13	1.5 ± 0.1	0.26 ± 0.06	
	α4W154 F <sub>3</sub> -Trp	8200 ± 100	360	0.63 ± 0.03	0.19 ± 0.01	
	α4W154 F <sub>4</sub> -Trp	12000 ± 2000	540	0.63 ± 0.04	0.23 ± 0.02	
Nic	Wild type	10 ± 1	-	1.7 ± 0.2	0.30 ± 0.04	
	α4W154 Trp	8.0 ± 1.0	0.80	1.5 ± 0.3	0.27 ± 0.01	
	α4W154 F-Trp	49 ± 2	4.9	1.5 ± 0.1	0.19 ± 0.01	
	α4W154 F <sub>2</sub> -Trp	110 ± 10	11	1.5 ± 0.2	0.26 ± 0.06	
	α4W154 F <sub>3</sub> -Trp	510 ± 60	51	1.1 ± 0.1	0.19 ± 0.01	
	α4W154 F <sub>4</sub> -Trp	1300 ± 100	130	1.1 ± 0.1	0.23 ± 0.02	
Cy	Wild type	3.9 ± 0.1	-	2.0 ± 0.1	0.24 ± 0.03	
	α4W154 Trp	3.8 ± 0.1	1.0	2.1 ± 0.1	0.17 ± 0.02	
	α4W154 F-Trp	15 ± 1	3.9	1.3 ± 0.1	0.19 ± 0.02	
	α4W154 F <sub>2</sub> -Trp	35 ± 2	9.0	1.1 ± 0.1	0.19 ± 0.02	
	α4W154 F <sub>3</sub> -Trp	78 ± 5	20	0.95 ± 0.04	0.20 ± 0.02	
	α4W154 F <sub>4</sub> -Trp	120 ± 10	30	0.88 ± 0.05	0.18 ± 0.02	
Var	Wild type	0.95 ± 0.02	-	1.7 ± 0.1	0.22 ± 0.03	
	α4W154 Trp	0.73 ± 0.02	0.77	1.4 ± 0.1	0.30 ± 0.03	
	α4W154 F-Trp	2.0 ± 0.2	2.1	1.1 ± 0.1	0.24 ± 0.01	
	α4W154 F <sub>2</sub> -Trp	2.4 ± 0.1	2.5	1.2 ± 0.1	0.21 ± 0.01	
	α4W154 F <sub>3</sub> -Trp	11 ± 1	12	1.0 ± 0.1	0.20 ± 0.02	
	α4W154 F <sub>4</sub> -Trp	15 ± 2	16	0.64 ± 0.04	0.18 ± 0.02	

**Supporting Table 2.** EC<sub>50</sub> values (nM), fold shift values, Hill coefficients (n<sub>H</sub>) and current size at +70mV (normalized to current size at -110mV). As described previously (Xiu et al., 2009), we interpret an I<sub>norm</sub> value below 0.10 as corresponding to an essentially pure population of A2B3 receptors and values at or above 0.20 as corresponding to an essentially pure population of A3B2 receptors. All receptor studies reported herein contain a L9'A mutation in the α subunit. a. Previously reported (Xiu et al., 2009). b. Previously reported (Blum et al., 2010). Errors are SEM.

<b>A2B3 Receptor</b>					
<b>Agonist</b>	<b>Mutation</b>	<b>EC<sub>50</sub> (nM)</b>	<b>Fold Shift</b>	<b>n<sub>H</sub></b>	<b>I<sub>norm</sub> (+70mV)</b>
ACh	α4T155 Thr <sup>a</sup>	410 ± 20	-	1.4 ± 0.1	0.044 ± 0.007
	α4T155 Tah <sup>a</sup>	370 ± 20	0.90	1.3 ± 0.1	0.02 ± 0.01
	β2L119 Leu <sup>b</sup>	440 ± 20	-	1.3 ± 0.1	0.04 ± 0.01
	β2L119 Lah <sup>b</sup>	3000 ± 100	6.8	1.2 ± 0.1	0.04 ± 0.01
Nic	α4T155 Thr <sup>a</sup>	90 ± 10	-	1.6 ± 0.1	0.044 ± 0.007
	α4T155 Tah <sup>a</sup>	1700 ± 100	19	1.2 ± 0.2	0.02 ± 0.01
	β2L119 Leu <sup>b</sup>	120 ± 10	-	1.5 ± 0.1	0.05 ± 0.01
	β2L119 Lah <sup>b</sup>	800 ± 30	6.7	1.3 ± 0.1	0.06 ± 0.01
Cy	α4T155 Thr	15 ± 1	-	1.2 ± 0.1	0.026 ± 0.009
	α4T155 Tah	130 ± 9	8.7	1.2 ± 0.1	0.03 ± 0.01
	β2L119 Leu	8.7 ± 0.5	-	1.2 ± 0.1	0.06 ± 0.02
	β2L119 Lah	540 ± 30	62	1.0 ± 0.1	0.06 ± 0.01
Var	α4T155 Thr	2.2 ± 0.1	-	1.3 ± 0.1	0.020 ± 0.002
	α4T155 Tah	30 ± 2	14	1.2 ± 0.1	0.029 ± 0.006
	β2L119 Leu	2.6 ± 0.2	-	1.3 ± 0.1	0.06 ± 0.01
	β2L119 Lah	4.7 ± 0.2	1.8	1.3 ± 0.1	0.05 ± 0.01

Supporting Table 2, continued

A3B2 Receptor					
Agonist	Mutation	EC <sub>50</sub> (nM)	Fold Shift	n <sub>H</sub>	I <sub>norm</sub> (+70mV)
ACh	α4T155 Thr	20 ± 1	-	1.4 ± 0.1	0.20 ± 0.02
	α4T155 Tah	25 ± 2	1.3	1.2 ± 0.1	0.22 ± 0.01
	β2L119 Leu	26 ± 1	-	1.6 ± 0.1	0.23 ± 0.02
	β2L119 Lah	220 ± 10	8.5	1.2 ± 0.1	0.24 ± 0.03
Nic	α4T155 Thr	9.9 ± 0.5	-	1.7 ± 0.1	0.20 ± 0.02
	α4T155 Tah	210 ± 20	21	1.6 ± 0.2	0.22 ± 0.01
	β2L119 Leu	12 ± 1	-	1.6 ± 0.1	0.23 ± 0.02
	β2L119 Lah	67 ± 3	5.6	1.4 ± 0.1	0.20 ± 0.03
Cy	α4T155 Thr	3.6 ± 0.4	-	1.4 ± 0.1	0.20 ± 0.04
	α4T155 Tah	96 ± 6	27	1.1 ± 0.1	0.19 ± 0.02
	β2L119 Leu	3.6 ± 0.1	-	1.9 ± 0.1	0.32 ± 0.03
	β2L119 Lah	51 ± 2	14	1.4 ± 0.1	0.24 ± 0.02
Var	α4T155 Thr	0.47 ± 0.03	-	1.5 ± 0.1	0.27 ± 0.02
	α4T155 Tah	8.9 ± 0.3	19	1.2 ± 0.1	0.23 ± 0.04
	β2L119 Leu	1.0 ± 0.1	-	1.5 ± 0.1	0.23 ± 0.03
	β2L119 Lah	1.1 ± 0.1	1.1	1.2 ± 0.1	0.22 ± 0.01

**Supporting Table 3.** EC<sub>50</sub> values (nM), fold shift values and Hill coefficients (n<sub>H</sub>) for S-MPP. All receptor studies reported herein contain a L9'A mutation in the α subunit. a. Previously reported (Blum et al., 2010). Errors are SEM.

S-MPP				
Receptor	Mutation	EC <sub>50</sub> (nM)	Fold Shift	n <sub>H</sub>
A2B3	Wild type <sup>a</sup>	11000 ± 400	-	1.7 ± 0.1
	β2L119 Leu <sup>a</sup>	14000 ± 900	1.3	1.5 ± 0.1
	β2L119 Lah <sup>a</sup>	1100 ± 40	0.08	1.5 ± 0.1
A3B2	Wild type	4500 ± 100	-	1.1 ± 0.1
	β2L119 Leu	4200 ± 300	0.93	1.6 ± 0.1
	β2L119 Lah	130 ± 10	0.03	1.2 ± 0.1

#### References.

- Blum AP, Lester HA and Dougherty DA (2010) Nicotinic pharmacophore: The pyridine N of nicotine and carbonyl of acetylcholine hydrogen bond across a subunit interface to a backbone NH. *Proceedings of the National Academy of Sciences of the United States of America* **107**(30):13206-13211.
- Xiu XA, Puskar NL, Shanata JAP, Lester HA and Dougherty DA (2009) Nicotine binding to brain receptors requires a strong cation-pi interaction. *Nature* **458**(7237):534-U510.